A LONGITUDINAL STUDY OF COMMUNITY-ASSOCIATED METHICILLIN-RESISTANT

STAPHYLOCOCCUS AUREUS COLONIZATION IN COLLEGE SPORTS PARTICIPANTS

By

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To my amazing parents, for being a great example and giving me unconditional support,

To Tomas, for brightening my days and supporting me through this journey

and

To God, for everything

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LIST OF ABBREVIATIONS

AAP   American Academy of Pediatrics

CA-MRSA   Community-associated methicillin-resistant *Staphylococcus aureus*

CDC   Centers for Disease Control and Prevention

CI   Confidence interval

GLM   Generalized linear model

GLMM   Generalized linear mixed model

HA-MRSA   Hospital-associated methicillin-resistant *Staphylococcus aureus*

HR   Hazard ratio

ICU   Intensive care unit

MRSA   Methicillin-resistant *Staphylococcus aureus*

MSSA   Methicillin-susceptible *Staphylococcus aureus*

NCAA   National Collegiate Athletic Association

NICU   Neonatal intensive care unit

OR   Odds ratio

PVL   Panton-Valentine Leukocidin

RR   Relative risk/Risk ratio

SA   *Staphylococcus aureus*

SCC*mec*   Staphylococcal cassette chromosome *mec*

SSTI   Skin and soft tissue infection

SPECIFIC AIMS

Staphylococcus aureus (SA) is the leading cause of invasive and skin and soft tissue infections in the United States. Community-associated methicillin-resistant *S. aureus* (CA-MRSA) has emerged as a common pathogen in the US and is associated with high morbidity and mortality. Approximately 30% of the general population harbors *S. aureus* in the anterior nares, [1-3] and nasal colonization is a known risk factor for staphylococcal infection. [1,2,4] Moreover, other body sites such as the oropharynx, axilla, and skin can also harbor these bacteria, [5,6] though the prevalence of colonization in these sites has not been as thoroughly studied. When assessed longitudinally, three different staphylococcal colonization profiles are recognized: persistent carriers (10-35% of population), intermittent carriers (20-75% of population), and non-carriers (5-50% of population).[2,5] These carriage profiles differ in their risk for infection, bacterial load, and molecular variability. [5,7] Among those that are colonized, some groups are at high risk for both colonization and disease, such as prisoners,[8,9] military recruits,[4] and athletes,[10,11 12] and are ideal models to study the longitudinal characteristics of SA colonization and disease.

Although knowledge of staphylococcal colonization has increased during the last few decades, the biology of nasal colonization is not completely understood, nor it is known why some colonized individuals do not develop disease. Most studies on at-risk populations, including athletes, have focused on nasal colonization only during an outbreak. It is likely that colonization in other body sites such as the oropharynx, axilla, and skin, as well as identification of the different colonization profiles, may be important in understanding the pathogenesis of infection. Finally, longitudinal data regarding colonization dynamics over time are scarce.

This study focuses on the analysis of a prospective cohort of college sports participants, assessing nasal and oropharyngeal MRSA colonization during the course of a two year period in order to

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define the dynamics of staphylococcal colonization over time and to identify potential strategies for prevention. To accomplish this, we performed an epidemiological study of SA colonization in athletes, assessing colonization in body sites other than the anterior nares and distinguishing between different colonization profiles. The <u>central hypothesis</u> of this study is that contact sports participants will differ in their staphylococcal colonization risk and patterns, as compared to those athletes who participate in noncontact sports. Three specific aims were proposed to test this hypothesis and achieve the overall objective of this study.

<u>Specific Aim #1</u>: To characterize the distribution of nasal and oropharyngeal colonization with *S. aureus* and community-associated methicillin-resistant *S. aureus* (CA-MRSA) in a prospective cohort of healthy collegiate student athletes over two academic years.

We hypothesized that the prevalence of both nasal and oropharyngeal colonization with *S. aureus* in general, and MRSA in particular, would fluctuate over time, depending on the timing within each team's athletic season, frequency of infection outbreaks, and antibiotic use. We also hypothesized that sports with greater direct contact between athletes would have a higher prevalence of staphylococcal colonization, as compared to more individualized sports. Therefore, staphylococcal colonization was assessed in over 300 healthy college athletes who were followed each month for two academic years. Defining the dynamics of *S. aureus* colonization over time will allow for improved surveillance of similar at-risk groups and provide new information about the longitudinal epidemiology of staphylococcal carriage.

Sub-Aim #1.1: To estimate the seasonal prevalence of staphylococcal carriage both in the general cohort and among members of the football team. Both the athletic and yearly seasons were assessed. We hypothesized that staphylococcal colonization prevalence would fluctuate over time, depending on each team's athletic season.

Sub-Aim #1.2: To estimate the time to colonization with *S. aureus* in new team members.

We hypothesized that new members of contact sports teams would become colonized more quickly than those in noncontact sports. The American Academy of Pediatrics (AAP) classifies sports as contact sports, limited-contact sports, and noncontact sports as follows:

> "In contact sports (e.g., basketball and soccer), athletes routinely make contact with each other or with inanimate objects […]. In limited-contact sports (e.g., softball and squash), contact with other athletes or with inanimate objects is infrequent or inadvertent. […] in noncontact sports (e.g., power lifting), […] contact is rare and unexpected, […]." [13]

For this study, sports were dichotomized into contact and noncontact, given that only one sport (baseball) is classified by the AAP as limited-contact. Baseball will be classified as noncontact, to reflect the less aggressive nature of the sport, as compared to contact sports. Other sports were classified following the AAP classification. [13] Football, lacrosse, soccer and basketball teams were classified as contact sports. Baseball, bowling, golf, cross-country, swimming, tennis, and track-and-field were classified as noncontact sports.

Sub-Aim #1.3: To assess whether prevalence of colonization differs by sub-types of CA-MRSA isolates and the virulence factors they possess.

We hypothesized that MRSA isolates that possess a specific repertoire of molecular characteristics would be differentially able to cause colonization. Detailed molecular data on the types of MRSA colonization isolates and their virulence factors was analyzed.

Sub-Aim #1.4: To describe the effect of infection outbreaks on the prevalence of MRSA colonization among the football team.

We hypothesized that prevalence of MRSA colonization would fluctuate in association with an outbreak and would likely decrease after decolonization or improved hygiene measures were instituted.

> "An outbreak of MRSA is an increase in the rate of MRSA cases or a clustering of new cases due to the transmission of a single microbial strain […]. The definition of *case* encompasses both newly infected and colonized patients. […] Clustering is defined as two or more cases closely related by time, location, or other epidemiologic linkages. […] An increase in the case rate can be defined […] experientially […] by an increase over the threshold or in the absolute number of cases. The threshold may be based on the MRSA baseline data for an individual institution or of the average baseline data from similarly sized institutions" [14]

Specific Aim #2: To model the association between colonization with CA-MRSA and the type of sport that athletes participate in, demographic characteristics, medical history, and CA-MRSA strain type, while adjusting for potential confounders. Effect measure modification by gender was also evaluated. Colonization was defined as no colonization, colonization with methicillin-susceptible *S. aureus* (MSSA) or colonization with MRSA.

We hypothesized that participating in contact sports would be associated with a higher frequency of staphylococcal colonization, as compared to noncontact sports. Therefore, baseline information on demographics, medical history and team membership was used to evaluate their association with subsequent staphylococcal colonization in a cohort of over 300 healthy college athletes, who participate on contact or noncontact sports, who were followed each month for two academic years.

Specific Aim #3: To model persistent staphylococcal colonization as a function of type of sport, site of colonization, and specific staphylococcal virulence factors, while adjusting for potential confounders. Persistent colonization was defined as colonization with *S. aureus* on ≥80% of at least 7 samplings, and it was compared to intermittent colonization and no colonization, which

were defined, respectively, as colonization on one or more of the samplings but less that 80%, and as never colonized.[3]

We hypothesized that people who are persistently colonized with *S. aureus* differ from those who are only intermittently colonized or not colonized, either in personal characteristics or in the type of isolate with which they are colonized. Therefore, we assessed whether an association exists between demographics, medical history, team membership, site of colonization and type of colonizing isolate, and whether athletes enrolled in the cohort were persistently colonized or not.

Sub-Aim #3.1: To model the association between persistent staphylococcal colonization in athletes and the type of sport they participate in, demographic and medical history elements, as compared to intermittent colonization or no colonization with *S. aureus*, while adjusting for potential confounders. We hypothesized that contact sports participants would be more likely to be persistent staphylococcal carriers due to more frequent and more intense exposures than noncontact sports participants.

Sub-Aim #3.2: To model the association between persistent staphylococcal colonization in athletes and site of colonization, while adjusting for potential confounders. We hypothesized that those athletes colonized with any type of *S. aureus* in both the nose and oropharynx would be more likely to be persistently colonized than those colonized with only *S. aureus* in their nose or oropharynx, or those who are not colonized.

Sub-Aim #3.3: To model the association between persistent staphylococcal colonization and the molecular characteristics of MRSA isolates obtained from collegiate athletes. We hypothesized that MRSA isolates that possess a specific repertoire of molecular characteristics would be differentially able to cause persistent rather than intermittent colonization.

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CHAPTER I

BACKGROUND

Staphylococcus aureus is the leading cause of skin and soft tissue infections, bacteremia, and osteoarticular infections. [15] Approximately one third of the general population carries *S. aureus* in the anterior nares at any given time. [1-3] In recent years, however, methicillin-resistant *S. aureus* (MRSA) has become the most frequently encountered infection-causing clone of *S. aureus* in many communities. [16-18]

Epidemiology of MRSA

MRSA causes significant morbidity and mortality in the general population

Approximately 100,000 invasive infections and 20,000 deaths per year occur due to methicillin-resistant *Staphylococcus aureus* (MRSA) in the United States. [15] MRSA is more virulent than MSSA, and patients with MRSA have worse outcomes than those with MSSA.[4,19-21] MRSA bacteremia in hospitals is associated with increased mortality, and a 1.2–2.0-fold increase in length of hospital stay and hospitalization costs, compared to bacteremia caused by methicillin-susceptible *S. aureus* (MSSA).[20,22] MRSA has become an increasing public health problem, particularly because it is no longer confined to health care institutions. [15,21,23] Though skin and soft tissue infections (SSTIs) due to MRSA are not usually fatal, they tend to cause recurrences[24,25] and can be severe enough to require hospitalization along with parenteral antibiotics.[26,27] Hospitalizations, in turn, represent higher costs as well as lost days of work or school.

The epidemiology of MRSA has changed

For many years, MRSA infections occurred only among hospitalized patients or among those with certain risk factors associated with MRSA colonization or infection in the hospital, such as having diabetes, hemodialysis, surgery, or certain immunodeficiencies.[21,28-34] More recently, however, MRSA infections have been described in previously healthy individuals without known risk factors for MRSA. [21,33]

Today, two broad categories of MRSA are recognized: hospital-associated MRSA (HA-MRSA), considered one of the most important causes of nosocomial infections around the world, especially in intensive care units (ICUs); [35,36] and community-associated MRSA (CA-MRSA), which has become the leading cause of skin and soft tissue infections. [37]

Community-associated MRSA (CA-MRSA) was first reported in Detroit in 1981 among injection drug users, and since then, CA-MRSA infections in otherwise healthy individuals have become increasingly common. [15,23] Documented outbreaks have occurred in American Indian and Alaska Natives, sports teams, prison inmates, soldiers, and child care attendees. [15,21,37,38] CA-MRSA is now the predominant cause of community-acquired staphylococcal infections, which is also occurring in hospitals. [21]

CA-MRSA colonization rates have been increasing

Approximately 1% of the general population harbors MRSA in the anterior nares at any given time. [10] According to data from a representative sample of the US population from the National Health and Nutrition Examination Survey (NHANES), as well as from a review of the literature, the prevalence of overall nasal colonization with *S. aureus* has decreased over time, from 32.4% in 2001-2002 to 28.6% in 2003-2004,[39] and from around 35% in 1934 to around 27% in the 2000s.[5] Despite the decrease in overall *S. aureus* colonization, MRSA colonization prevalence

has increased over time, from 0.8% to 1.5%.[39] Not only has the proportion of colonizing MRSA increased, but the percentage of infections caused by USA300 MRSA isolates, which is the epidemic MRSA clone in the United States, has also increased, from 8% in 2001 to 2002 to 17.2% in 2003 to 2004.[40]

Prevalence of MRSA colonization in pediatric populations has also increased over time.[16] In 2004, a study performed by our group found an MRSA nasal colonization prevalence of 9.2%,[16] which was significantly higher than the 0.8% reported in 2001 in a similar pediatric population at the same institution.[41]

Nasal carriage of *Staphylococcus aureus* increases the risk of infection

The first report of an association between nasal staphylococcal colonization and infection was written in 1931.[5] Since then, several studies have confirmed that carrying *S. aureus* in the anterior nares increases the risk of subsequent staphylococcal infection,[1,4,42,43] and that a majority of the infections were caused by the same strain that was colonizing the patient.[42,43] In a study of non-surgical patients, Wertheim *et al* reported that the risk of nosocomial *S. aureus* bacteremia was three times higher
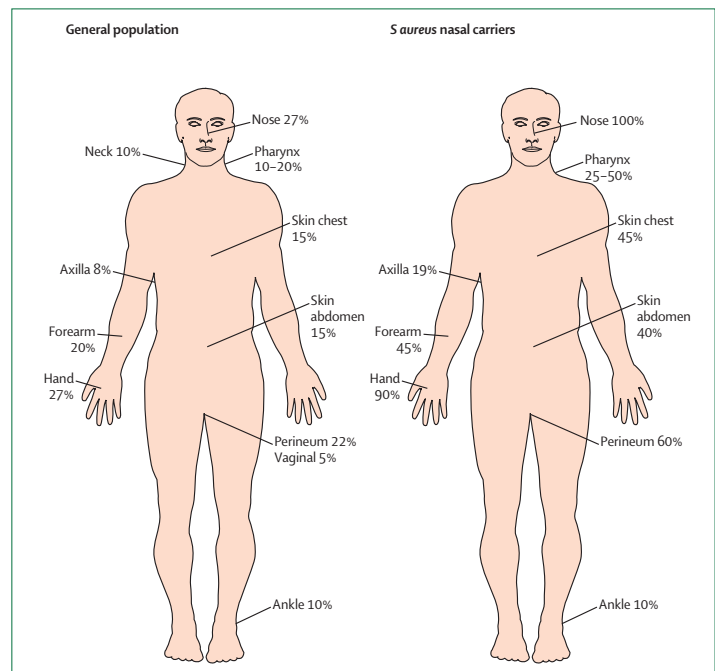
Figure 2: S aureus carriage rates per body site in adults
There is an increase in carriage rates at extra-nasal sites within nasal S aureus carriers. The mentioned rates are approximations using data from the literature cited in the text.

Figure 1: Taken from Wertheim HF, *et al*. *Lancet Infect Di*s Dec 2005; 5(12): 751-762.

for *S. aureus* carriers than for non-carriers (relative risk 3.0; 95% CI 2.0-4.7). Upon genotyping, they found that 80% of bacteremia-causing strains were identical to the strain isolated from the nares at the time of admission to the hospital. [43] Similarly, two studies conducted by von Eiff *et al* also comparing nasal *S. aureus* isolates with isolates from the blood of patients with *S. aureus* bacteremia found, in two separate studies, that isolates from 82.2% and 86% of the patients were clonally identical. [42]

Furthermore, the largest prospective study to date by Ellis *et al*, in a cohort of soldiers, was the first to describe that the attack rate of skin and soft tissue infections (SSTIs) for those colonized with MRSA was higher than for those colonized with MSSA. [4] The prevalence of CA-MRSA nasal colonization was 3%, and 28% for MSSA. [4] However, 38% of those colonized with CA-MRSA developed SSTIs, as compared to only 3% of those colonized with MSSA. [4]

The high prevalence of nasal staphylococcal colonization is another reason for concern.
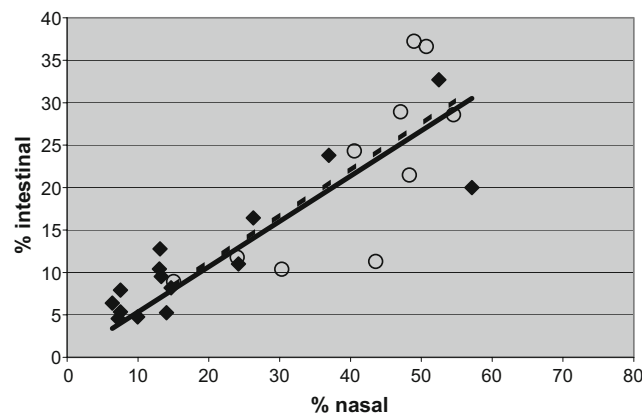
Fig. 1 Relation between frequencies of nasal and intestinal carriage for *S. aureus* (*circles*) and for MRSA (*diamonds*). Lines show the linear regression of *S. aureus* (*dotted line*) and MRSA (*straight line*), the slope of the linear regression lines are 0.55 and 0.53, and R^2 are 0.6012 and 0.7381 for *S. aureus* and MRSA respectively. The plotted data for *S. aureus* are extracted from: [3, 10, 17–19, 24, 44, 48, 77, 90] and for MRSA from: [10, 18–21, 23, 24, 27, 31, 33, 36, 38, 39, 51, 91]

Figure 2: Taken from Acton DS, *et al. Eur J Clin Microbiol Infect Dis* 2009; 28:115-127.

Approximately 30% of the general population carries SA at any given time, [1-3,39] and about 1.5% carry MRSA. [10,39] Though *S. aureus* can colonize other body sites such as oropharynx and skin, the nose has been the most studied colonization site, and the one yielding the highest colonization rates (Figure 1). [44] In a recent study by Lauderlade *et al*, the authors obtained surveillance cultures from the nares, throat or

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sputum, axilla and perineum on patients upon admission to an ICU. [45] They reported that cultures from the nares alone detected 72.5% of all colonized patients. When combining nares and throat cultures, they detected 85.4% of colonized patients. [45] Furthermore, the highest load of MRSA was found in the nares, followed by throat, then perineum, while the fewest colonies were found in the axilla.[45] Similarly, Schechter-Perkins *et al* obtained nares, oropharynx, palms, groin, perirectal wounds and catheter insertion sites surveillance cultures from patients presenting to an Emergency Department. [46] They found a 23% prevalence of MSSA nasal colonization and 17% prevalence of exclusively extra-nasal MSSA colonization, whereas 3% carried nasal MRSA and 2% carried MRSA exclusively in other sites.[46] Nonetheless, they report that for both MSSA and MRSA, the most commonly colonized extra-nasal site was the oropharynx, with a prevalence of 22% for MSSA and 3% for MRSA.[46] However, another recent study suggested that only looking at nasal colonization might underestimate colonization prevalence and risk of infection. [6] This study found that rectal, rather than nasal carriage of USA300, could be potentially associated with an increased risk of SSTIs in a pediatric population, though it was not statistically significant (OR 5.22; 95% CI 0.87-31.32).[6] Acton *et al*, on the other hand, on a review of the literature on intestinal or perineal carriage of *S. aureus*, reported that the detection of intestinal carriage in healthy subjects was half of that for nasal carriage, and also found a positive correlation between the nasal and intestinal carriage for *S. aureus* and for MRSA (Figure 2).[47] Thus, the role of extra-nasal and nasal colonization on the epidemiology and pathogenesis of infection is still unresolved.

Different *S. aureus* colonization profiles confer different risk of infection

Three different *S. aureus* carrier patterns are recognized. [1,2,44] Some people carry SA nearly persistently, and thus are called persistent carriers. This group represents around 20% (10-35%) of the population. [1,2] Another group, around 60% (20-75%) of the population, are intermittently colonized and are called intermittent carriers. [1,2] A third group, representing around 20% (5-50%) of the population, are rarely colonized and thus are called non-carriers.[1,2] Those who are

staphylococcal carriers during ≥80% of the times at which they are sampled are classified as persistent carriers. Those who are never colonized are non-carriers, and those colonized at least once, but <80% of the times at which they are swabbed, are classified as intermittent carriers.[1,3,48] Identification of these profiles has been done by obtaining samples from participants ranging from once per week to once every three to four weeks.[3,7,49]

These three carrier patterns differ in ways other than the frequency with which they are colonized with SA. Persistent carriers are typically colonized with a single strain of SA. [2] Some studies have shown that, after decolonization and artificial inoculation with a mix of SA isolates, persistent carriers tend to become re-colonized with their original colonizing strain. [7,49] Intermittent carriers and non-carriers, on the other hand, can have different colonizing strains across time. [7,49] Furthermore, persistent carriers seem to be at increased risk of infection when compared to intermittent or non-carriers. Additionally, persistent carriers have been shown to have a higher bacterial load in their nares than intermittent or non-carriers. [3,7] This higher load has been a suggested explanation for persistent carriers' increased risk of infection.

Given the distinctions between these three colonization types, it is necessary to identify and differentiate between carriers in order to conduct a thorough examination of colonization patterns, the epidemiology of staphylococcal colonization and its role on the pathogenesis of infection. However, few studies have focused on differentiating among these three carrier profiles. Furthermore, given the similarities between intermittent and non-carriers, some studies have suggested that these two profiles might in fact represent a single entity.[7]

Characteristics of MRSA

Clones of CA-MRSA have a specific set of virulence factors

Several clinical and molecular differences between HA-MRSA and CA-MRSA have been described. First, CA-MRSA carries type IV or V staphylococcal cassette chromosome *mec* (SCC*mec*), whereas HA-MRSA harbors types I-III. [15,33,36,37,50,51] The mobile genetic element known as SCC*mec* carries the methicillin resistance gene of MRSA strains. [52] SCC*mec* carries the *mec* gene complex, which contains the gene *mecA*, a chromosomal gene that codes for PBP2a in MRSA. [21,52-55] This mobile genetic segment is not native to *S. aureus*; rather, it originated from the coagulase negative staphylococci, [56,57] and it contains site-specific recombinases and invertases that provide it with the ability to insert itself into the genome of *S. aureus* at a very specific site, just downstream of the origin of replication. The SCC*mec* region is important to molecular epidemiologists because of the distinctions that are made between traditional hospital-associated MRSA and newly emergent community-associated MRSA (CA-MRSA). [50-52,58-61] SCC*mec* elements are commonly classified into types I, II, III, IV, and V. [62] SCC*mec* typing is required for complete MRSA characterization, and it has been incorporated into the new MRSA nomenclature by the International Union of Microbiology Societies. [58,62]

Another difference is that CA-MRSA tends to be resistant to fewer antimicrobial classes than HA-MRSA. [21,23,33,37] SCC*mec* types I, II, and III, present in HA-MRSA strains, are large pieces of genetic information (40-60 kb) and encode not only resistance to methicillin but to other antibiotics as well. Types IV and V, found in CA-MRSA, are smaller in size (24 kb) and lack these additional antibiotic resistance determinants. The result is that CA-MRSA strains carrying type IV or V SCC*mec* are capable of maintaining methicillin resistance but with less of a "fitness cost" than their hospital-acquired counterparts and are susceptible to more antibiotic agents. [63]

Third, several studies have also shown that CA-MRSA strains carry a range of virulence genes that are distinct from virulence genes in typical HA-MRSA strains. [21,33,37] HA-MRSA carries superantigenic exotoxins, such as the staphylococcal enterotoxin A. [36] CA-MRSA, on the other hand, carries Panton Valentine leukocidin (PVL), toxic shock syndrome toxin 1, exfoliative toxins and enterotoxins. [15,23,33,36,37] Fourth, CA-MRSA seems to preferentially infect children and young adults without risk factors for antibiotic-resistant organisms. [21,23,33] Finally, the spectrum of disease caused by CA-MRSA appears to be similar to that caused by methicillin-susceptible *S. aureus* (MSSA). [33] Infections caused by CA-MRSA range from minor skin and soft tissue infections to rapidly fatal, necrotizing pneumonia and overwhelming sepsis, though skin and soft tissue infections appear to be the most frequently reported presentation of CA-MRSA infections.[21,23,33,36]

The set of virulence factors present in CA-MRSA are thought to be necessary for colonization, maintenance and infection, and seem to be responsible for the range of disease it causes. Identifying the virulence factors present in colonizing- and infection-causing isolates might elucidate the factors' role and the isolate's potential for infecting different individuals. This knowledge could shed light on the pathogenesis of staphylococcal disease.

The *mecA* gene is responsible for methicillin resistance in *Staphylococcus aureus*
Methicillin is a semisynthetic beta-lactam antibiotic that was introduced in the late 1950s. [62,64] Penicillin had quickly become ineffective (*S. aureus* inactivates it with a specific enzyme), making methicillin a critical antibiotic for *S. aureus*. [52,64,65]However, in the 1960s, after introduction of semisynthetic penicillins, MRSA emerged as a nosocomial pathogen in several European countries and in the United States. [21,52,66-69] Although methicillin is no longer used in clinical practice, the term methicillin-resistant *S. aureus* (MRSA) continues to be used to describe *S. aureus* strains resistant to all penicillins.

Methicillin, like other beta-lactam antibiotics, acts by inhibiting the synthesis of bacterial cell walls by covalently binding to and competitively inhibiting the penicillin-binding proteins (PBPs), which catalyze reactions necessary for peptidogylcan synthesis. [70] The beta-lactams induce the synthesis of a new penicillin-binding protein, PBP2a, which has low affinity for beta-lactam antibiotics and, therefore, confers resistance to virtually all beta-lactam antibiotics. [52-54,71] All MRSA strains produce PBP2a. [52,72,73]

Is Panton-Valentine Leukocidin (PVL) a Major Virulence Factor in CA-MRSA?

PVL is a two-component cytotoxin, and was first described in 1932.[74] This toxin is codified by two genes, *lukS-PV* and *lukF-PV*.[74] These genes are present in many CA-MRSA strains; particularly, there is an association between USA300 strains and PVL. [37] PVL destroys leukocytes, and infections with PVL-carrying strains have been associated with necrotizing pneumonia,[75-77] dermonecrosis,[76] complicated osteomyelitis,[78] furunculosis and abscess formation, [76,79] but is less frequently expressed in colonizing strains. [4,80,81] In CA-MRSA stains, the PVL gene locus has been found in as many as 100% (117/117) of worldwide clinical isolates.[79,82-86] One study reported that 98% of abscess-causing isolates were PVL positive, and 97% of these were USA300.[6] However, PVL is less commonly found in nasal colonization isolates. In 2004, one study in a pediatric population reported that only 10 (22%) of the 46 carriage isolates were found to possess the PVL gene locus by polymerase chain reaction.[87] In the Ellis study of soldiers entering basic training, [4] all of the clinical wound isolates tested contained PVL; however, only 21 of the 36 nasal carriage isolates (58%) were PVL positive. Another study reported that 4% of MSSA and 62% of MRSA colonizing strains carried PVL, respectively, representing an overall prevalence of PVL of 11%.[46] Data from NHANES, reported a PVL prevalence of 14%.[39] These data suggest that certain strains of CA-MRSA might be more likely to be associated with infection than others, such as strains with PVL. However, whether PVL is critical in the pathogenesis of these clinical manifestations of staphylococcal disease remains

unclear. [88,89] It is crucial to identify virulence factors, such as PVL, present in colonizing and

infection-causing isolates. Increased knowledge about the presence of these factors in different

sets of isolates might elucidate the factors' role and the isolates' potential for infecting different

individuals. This knowledge would also help comprehend the sometimes poorly understood

pathogenesis of staphylococcal disease.

MRSA strain types are categorized by a variety of characteristics

While creating a national database of MRSA pulsed-field gel electrophoresis (PFGE) types,

McDougal and coworkers first described 8 distinct pulsed-field types, designating them USA100

through USA800. [90] They noticed that the different clusters of MRSA isolates not only differed in

their PFGE patterns, but also in their epidemiology. USA types USA100, -200, -500, -600, and -

800 were primarily from healthcare-associated infections, while isolates from community-onset

infections belonged to types USA300 and -400. [90] Isolates that belonged to USA700 belonged to

both community and healthcare associated infections. [90] More recently, other USA pulsed-field

types have been described (USA800 – 1200).[40]

Currently, USA300 is the major epidemic clone of CA-MRSA in the United States. [15,91] It has

been a major cause of both invasive disease and skin and soft tissue infections. [15,92] However, few

studies have analyzed the molecular epidemiology and the population dynamics of *S. aureus*

colonizing strains, particularly of CA-MRSA USA300 over a prolonged period of time. [1] A recent

study from our group showed that molecular characteristics and virulence factors present in

colonizing CA-MRSA strains are different than those present in strains that cause invasive and

non-invasive disease.[81] Of the infection-causing isolates, 82% were USA300 and 87% carried

PVL genes, compared to only 18% and 24% of colonizing isolates, respectively.[81]

MRSA and Athletes

Athletes have increased risk of CA-MRSA

Athletes, especially those who participate in contact sports, appear to be at increased risk of both colonization and infection with CA-MRSA. About 16 reports, corresponding to 35 different populations of athletes, have been published (See Appendix A). The majority were studies from the United States. Most studies, 12 in total, included a football population, while the next more commonly reported sports were wrestling, soccer, and basketball, accounting for 3 studies each. Most sports teams analyzed have been composed of males (n=23 studies), as compared to only 12 studies that included female teams. Also, most studies have included a collegiate athlete population (28 studies), 3 studies were from high school participants, 2 were from professional teams, and 2 were unspecified. However, a main limitation in the earlier studies has been that sample sizes are small, ranging from 1 to 147 per sport, which does not allow adequate statistical power to perform analyses beyond calculating the prevalence of colonization and incidence of infection. Fifteen of these studies have reported the prevalence of MSSA nasal colonization, while 34 reported the prevalence of MRSA nasal colonization. However, despite finding infections, some reports did not find nasal MRSA carriers.

Prevalence of colonization with MRSA is higher in athletes than in the general population. [10,93] The mean prevalence of MSSA colonization in athletes, assessed in my meta-analysis based on studies presented in Appendix A, was 38.6% (95% CI: 24.7 to 52.5%). The mean prevalence of MRSA colonization varied according to how standard errors were estimated (since some studies reported zero prevalence for MRSA colonization), and ranged from 0.9% to 4.5%. In an earlier reported pilot study from our group, however, the prevalence of MRSA colonization in our athletes was higher than the average colonization rates in athletes, and rates varied throughout the athletic seasons.[94] Up to 16.5% of the members of the football team carried nasal MRSA during

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the football season, which represented a significant increase from the 4.4% prevalence during the off-season (Figure 3).[94]

Several factors have been proposed to contribute to this increased risk. Some of these factors include direct skin-to-skin contact [95], frequent skin abrasions (turf burns), [11,25,96] sharing personal items such as towels, soap, [25,95] uniforms, [97] and water bottles; football player position, [11,96] and contact with contaminated surfaces in weight rooms and locker rooms. [98] There has not been, however, a consistent set of risk factors associated with staphylococcal carriage and infection in athletes, since some studies failed to show significant associations for some of the previously mentioned risk factors.
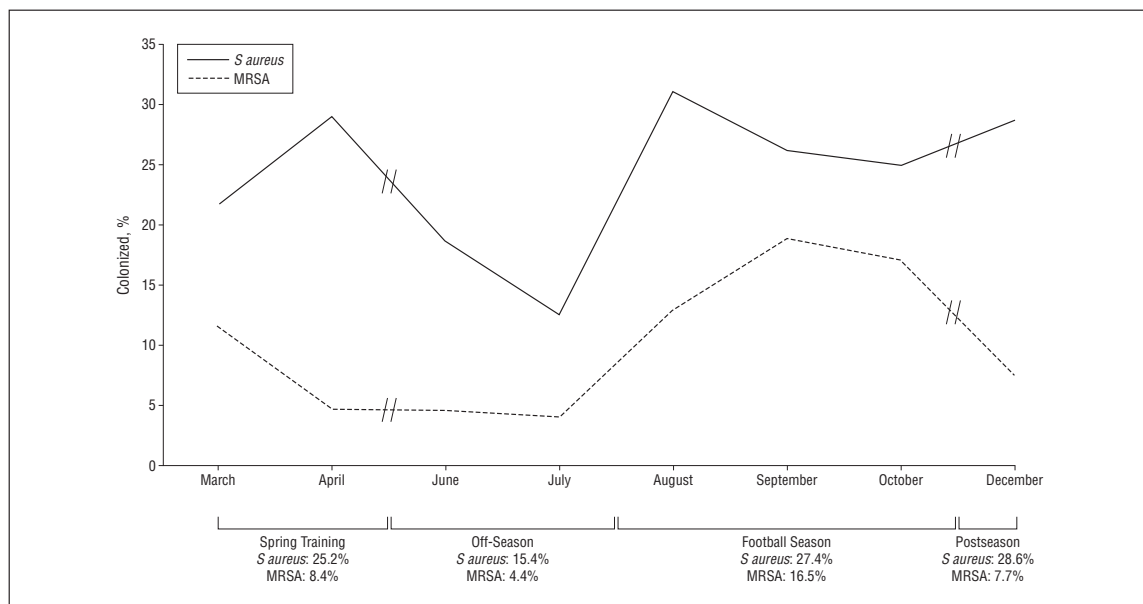
Figure 1. Staphylococcal colonization (by month) in a men's collegiate football team. Monthly cultures are divided into spring training (March/April), the off-season (June/July), the regular football season (August-October), and the postseason (December). Monthly results are expressed graphically while aggregate colonization rates are expressed below each season. Double hash marks represent months in which nasal swabs were not performed. The frequency of methicillin-resistant *Staphylococcus aureus* (MRSA) colonization during the football season was significantly higher than in spring training (16.5% vs 8.4%; *P*=.003), the off-season (16.5% vs 4.4%; *P*=.004), and the postseason (16.5% vs 7.7%; *P*=.04).

Figure 3. Taken from Creech *et al*. *Arch Pediatr Adolesc Med*. 2010;164(7):615-620

The role nasal colonization plays in the pathogenesis of infection in athletes remains unclear. Several investigators report extremely low rates of nasal MRSA carriage, despite the occurrence

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of an outbreak.[11,96] Furthermore, despite multiple MRSA outbreak reports in athletes, surveillance studies of colonization patterns during a non-outbreak setting are lacking. Thus, the understanding of nasal colonization, and strategies aimed at management and prevention of CA-MRSA outbreaks in sports remain unclear.

MRSA outbreaks have affected the athletic community

MSSA outbreaks in athletes have been previously reported. Bartlett *et al*, in 1982, had reported an outbreak of *S. aureus* furuncles among 26 players of a high school football team.[99] In 1989, Sosin *et al* reported a similar outbreak in 31 players of the football and basketball teams in a high school.[100] However, since the first documented CA-MRSA outbreak occurred in athletes in 1993,[101] outbreaks, especially in wrestlers, football teams, [11,25,102] and rugby teams [103] have been reported. Though outbreaks in other sports have been documented, [93,104] the most affected seem to be contact sports. The first MRSA outbreak among an athletic team occurred in a high school wrestling team.[101] The attack rate for the team was 21.9%, and ranged from 0% among 12th graders to 33% among ninth graders.[101] Upon analysis, however, no risk factors for MRSA infection were found, and only one individual had nasal MRSA colonization.[101]

In 2003, the CDC reported 4 clusters of MRSA SSTIs among athletes. There were 5 cases among fencers in Colorado, with 60% being female, and 60% requiring hospitalization and intravenous antibiotics. [97] Possible sources of exposure might have been sharing of equipment, masks and clothing. Also, 10 members of a college football team in Pennsylvania and 2 members in Los Angeles presented with SSTIs, requiring 7 and 2 in each outbreak to be hospitalized, respectively.[97] Possible risk factors for infection were skin trauma and sharing of towels and lotions.[97] In each setting, isolates obtained from infected patients were indistinguishable. Nonetheless, colonization was not assessed in these populations.

Similarly, in 2004, Begier *et al* reported an outbreak in a college football team, with an attack rate of 10% among 100 team members. Isolates from 6 of the 10 infections, and 3 colonizing isolates from 2 hospitalized patients were indistinguishable, USA300 isolates, with SCC*mec* IVa and PVL positive.[96] Of the players, 44% had nasal MSSA colonization, but none had nasal MRSA. This study reported, on univariate analyses, an increased risk of infection for cornerbacks and wide receivers (relative risk [RR] =17.5 and 11.7, respectively), for those who had turf burns (RR=7.2), or shaved their bodies (RR=6.1), and for sharing a whirlpool more than twice per week (RR=12.2).[96] This study supported the hypothesis that MRSA could spread by direct contact between players, and that transmission was facilitated by injury to the skin.

A similar outbreak occurred during 2003 in the Saint Louis Rams professional football team, with an attack rate of 9%.[11] Though no MRSA was obtained from nasal or environmental samples, 42% of players and staff had nasal colonization with MSSA, and MSSA was also isolated from whirlpools and taping gel.[11] Linemen or linebacker positions had a relative risk for infection of 10.6. All infecting isolates were indistinguishable, including isolates from an opposing team that, after playing the Rams, also suffered an outbreak of MRSA. These isolates belonged to a novel clone subtype, USA300-0114, which contained PVL and SCC*mec* IVa.[11] This outbreak resulted in a total of 17 missed days of practice and games.

Most reported outbreaks thus, have been caused by bacterial strains with molecular characteristics consistent with CA-MRSA. These strains have been type USA300, they carry PVL, and the staphylococcal cassette chromosome *mec* type IVa. [11,25,96,102] These outbreaks have caused skin and soft tissue infections, with high morbidity, high treatment and hospitalization costs, and loss of play for affected team members. Thus, elucidating which individuals might be at higher risk of infection, and which colonizing isolates tend to cause more infections is crucial for implementing

better prevention and management strategies, not only for the athlete population, but for the general population as well.

Several interventions have been used to control MRSA outbreaks in athletes

The CDC and other sports associations have established guidelines for preventing transmission and infection with CA-MRSA in sports participants. [97]After several clusters of CA-MRSA infections occurred among athletes, including fencers, football players and wrestlers, the CDC published a set of guidelines, focused on good hygiene, both personal and environmental. [97] Guidelines indicate that emphasis should be placed on covering wounds appropriately, avoiding shared personal items like towels or soap, disinfecting shared equipment frequently and properly, and educating athletes and trainers to report any suspicious skin lesion and cover and treat it appropriately to avoid transmission to other team members. [97] Furthermore, the athletic facilities needed to be clean, including clean towels, soap and hot water in showers. [97]

Treatment with mupirocin nasal ointment has been implemented in sports teams' outbreaks. The decolonizing effect of mupirocin, however, seems to be only transient; it is not effective for long term CA-MRSA prevention. [105] Furthermore, the potential for development of bacterial resistance exists. [105] Others have used hygiene education, along with daily hexachlorophene showers. [25] However, education using the CDC guidelines [97] seems to be the most widely used intervention.[93]

Sanders *et al*, 2009 [106] implemented a cost-effective, non-invasive, non-pharmacological intervention to educate members of a collegiate football team in a non-outbreak setting. The intervention, called Training CAMP Program, consisted of two components. First, education of the football team was done before the beginning of the season by presenting the most current guidelines for prevention, early detection, appropriate care and treatment options of CA-MRSA infections. Additionally, to ensure knowledge was retained, all players and trainers were given a

booklet containing the CDC guidelines, and posters by the CDC were placed in athletic areas.

Knowledge grained at the information session was measured by administering a pre-test and a

post-test; the test was administered again after the end of the football season. The second

component was placing sanitizing wipes in athletic facilities. With this educational intervention,

the incidence of CA-MRSA infections decreased over 75% as compared to the previous year, and

this reduction resulted in healthcare savings of $4.51 to $11.29 for every dollar spent on the

intervention. [106]

Even though the CDC and NCAA have established guidelines for prevention of CA-MRSA

infections in athletes, these might not be sufficient when a cluster or outbreak develops. Although

different measures might work to eradicate such outbreaks in different circumstances and

populations, it is essential to establish universal guidelines and protocols for management of

colonization and infection with CA-MRSA. With such guidelines, all medical personnel,

regardless of their institution, could implement the same protocol in the case that the CDC and

NCAA guidelines for prevention are not sufficient to eliminate an outbreak. The proposed study

will help understand which risk factors and CA-MRSA isolates, associated with colonization and

infection, should be the main target during an intervention. Identification of risk factors and of

isolates associated with higher risk of infection would be a first step for developing consensus as

to which interventions should be implemented under specific circumstances.

Epidemiology of MRSA colonization and infection in athletes is poorly understood
Even though there is extensive information on CA-MRSA outbreaks in sports settings,

information about the baseline colonization rates is lacking. Only a few studies have looked at

staphylococcal colonization in athletes in a non-outbreak setting. [94,107,108] Some studies have

looked at staphylococcal carriage, but only during an outbreak, [11,25,96,102] and most outbreak

reports have failed to analyze staphylococcal colonization in the nares and other body parts. One

study performed pre-season surveillance cultures on a professional football team, and did not find MRSA nasal carriers, though 26.8% of nasal cultures grew MSSA.[107] During the football season, however, 5 cases of CA-MRSA SSTIs occurred, suggesting that screening with a single nasal swab was not sensitive enough to detect MRSA carriers. In a previous pilot study on college football and lacrosse teams, we found that colonization rates varied during the athletic seasons, with the lowest prevalence of both *S. aureus* and MRSA nasal colonization during the football off-season (15.4% *S. aureus* and 4.4% MRSA), and the highest prevalence during the football season (27.4% *S. aureus* and 16.5% MRSA).[94]

Furthermore, information available on the molecular characteristics of baseline colonizing isolates is scarce. Previously mentioned studies have characterized the outbreak-causing strains mostly as USA300, PVL positive isolates, but only our pilot study that analyzed staphylococcal carriage in football and lacrosse collegiate teams has reported the molecular characteristics of the colonizing bacteria, as well as of the infection-causing bacteria, and the colonizing bacteria represented a very heterogeneous group of MRSA isolates. [94]

Additionally, nasal carriage of MRSA does not seem to be a sufficient risk factor for the development of infection. [94] Thus, other bacterial or host factors must be in play for infection to occur. The identification of such risk factors would help find individuals who are at higher risk of infection, and subsequently, implement interventions appropriate for prevention of infection and transmission to fellow teammates. Furthermore, understanding the dynamics of staphylococcal colonization and infection in this higher risk population is a step forward to understanding the pathogenesis and epidemiology in the general population.

CHAPTER II

RESEARCH STRATEGY

Research Methods and Design

Study Overview

From August 2008 to April 2010, all varsity student athletes at Vanderbilt University were invited to participate in the study. The primary purpose of the study was to examine the dynamics, epidemiology and risk factors for community-associated methicillin-resistant *S. aureus* (CA-MRSA) carriage. Additionally, the study aimed to assess variations in the frequency of staphylococcal colonization over time, and incidence of infection with CA-MRSA. Incidence rate of infection was defined as the number of infected subjects divided by the total person-time throughout the study. A baseline assessment of all participants was performed at the beginning of the study, along with a questionnaire to ascertain risk factors for colonization. Additionally, nasal and oropharyngeal swabs were obtained immediately after enrollment, and again monthly until the end of the study, to detect staphylococcal colonization. Athletes were monitored for signs and symptoms of skin and soft-tissue infections (SSTIs) and individuals were asked to report any new skin lesion to team physicians and study personnel, even if lesions were minor. When SSTIs developed, cultures of the infected sites were taken, when possible. After colonization or infection samples were collected, a molecular analysis of the obtained isolates was performed, focusing on characterization of the methicillin resistance gene (*mec*A) and the genomic region in which it is located (Staphylococcal Cassette Chromosome *mec*), the clonality of isolates as determined by repetitive sequence based PCR (rep-PCR), and on the presence of specific staphylococcal virulence factors, such as PVL.

Study Participants

Subjects were recruited from Vanderbilt University in Nashville, Tennessee, a privately funded university, with over 6,200 undergraduate students. Subjects were eligible if they were part of the Vanderbilt University Varsity Athletic Program and were over 18 years of age. Participants were recruited by study personnel, who were not related to team activities, to prevent possible coercion from coaches and training staff. All questions or concerns about the study were directed to study personnel. Coaches and training staff were not involved in the conduct of the study. From at least 430 eligible athletes, 377 subjects, including 12 trainers, were enrolled. Participation among athletes was 84.9%. Subjects were asked to remain in the study for two years, or until completion of undergraduate education, or until ending their involvement with the varsity athletic program.

All athletes were given a consent document that described the study and provided information about what participating in the study involved, and about potential risks and benefits from participating, so that individuals could make a well-informed decision about participating or not in the study. Further, it was explained to all subjects that participating in the study was voluntary, and that they could decide to withdraw at any moment, without it affecting their participation on the team. IRB-approved consent forms were signed by participants before they were subjected to any study-related procedure. Coaches and training staff were not allowed to obtain informed consent from subjects.

Subjects were asked to provide study personnel with specific health information, such as history of staphylococcal infections, current and recent antimicrobial use, and hospitalization for *S. aureus* infections. However, personal health information was not accessed through evaluation of the medical record or communication with subjects' personal physicians. This was to ensure that only personal health information relevant to conducting the proposed research was available for review. All source documents containing information about the subjects are kept in locked file

cabinets in the offices of the Vanderbilt Vaccine Research Program. In addition, databases

created for the purpose of duplication of records and for statistical analysis were maintained in

the Vanderbilt Research Data Capture System (REDCap)[109] and electronic files derived from the

database were protected in password-protected files on password-protected computers.

Specimen Collection and Microbiology Procedures

Nasal and Oropharyngeal Cultures

Nasal and oropharyngeal samples were obtained at the beginning of the study to assess baseline

carriage status, and at each monthly follow-up visit. A total of 3,291 nasal and oropharyngeal

samples were obtained from 377 subjects over 18 sampling periods. Samples were collected using

Culturette™ swabs (BD Diagnostic Systems). The swab was moistened with sterile Amies liquid

media prior to inserting it into each naris, and then rotated along the inside of both nares for 5

seconds. Oropharyngeal swabs were not pre-moistened with sterile media. Swabs were stored at

room temperature until primary plating. Within 12 hours of collection, the swab was placed in 5

mL of tryptic soy broth (TSB) with 6.5% NaCl and incubated at 37°C for 24 hours.

Isolation of *S. aureus*

After the enrichment step in TSB, 100 µL of the bacterial suspension were plated onto bi-plates

of mannitol salt agar, with and without 6 µg/mL of oxacillin (MSA, MSA-ox, Remel). All plates

were incubated for 48 hours at 37°C, an additional 24 hours at room temperature, and then

inspected for yellow colonies indicative of mannitol fermentation, which is a characteristic of *S.*

aureus. After sub-culturing onto blood agar plates, and incubating them for 24 hours at 37°C,

coagulase testing was performed on all isolates (Staphaurex Plus, Remel), and coagulase-positive isolates were stored for further characterization.

Confirmation of methicillin-resistant *S. aureus* (MRSA)

Isolates recovered from the mannitol salt agar plates containing 6 µg/mL of oxacillin (MSA-ox) that were coagulase positive were considered potential methicillin-resistant *S. aureus* (MRSA) strains. Bacterial DNA from potential MRSA isolates was purified and tested by polymerase chain reaction (PCR), using a commercial kit (MoBio), for the presence of the *mec*A gene that encodes the altered penicillin-binding protein 2a (PBP2a) using previously described methods. [16]

Determination of Molecular Characteristics of MRSA Isolates

Determination of Staphylococcal Cassette Chromosome *mec* (SCC*mec*) type

Genomic DNA from each isolate was used for a multiplex PCR that includes eight loci labeled A through H per the protocol of Oliveira and de Lancastre. [58] PCR products were resolved in a 2% agarose gel in 1x Tris-borate-EDTA buffer at 100V and visualized with ethidium bromide. Assignment of SCC*mec* type was based upon characterization of banding patterns.

Determination of Overall Genetic Relatedness: Repetitive Sequence Based PCR (rep-PCR)

The DiversiLab System (bioMerieux, Inc) was used to establish the relatedness of strains by detecting differences within the entire genome of *S. aureus* strains. Staphylococcal DNA was extracted and purified. Using commercially available staphylococcal oligonucleotide primers that bind to specific repetitive sequences interspersed throughout the genome, multiple fragments of

variable lengths are amplified. These fragments are separated using microfluidic electrophoresis and fluorescent intensity was measured and graphically displayed.

Rep-PCR analysis

DiversiLab reports are generated through a web-based server maintained by bioMerieux. Dendrogram analysis, providing a hierarchical cluster representation of similarities between samples, was performed in a similar fashion to that developed by Tenover *et al* in the analysis of pulsed-field gel electrophoresis band patterns. [90,92] In addition, we generated similarity matrices, graphical overlays, and two-dimensional scatterplot cluster analyses in order to compare local strains to each other as well as to an internationally representative staphylococcal reference library. This analysis also served to identify the USA type of each isolate.

Detection of Panton-Valentine Leukocidin (PVL)

Genomic DNA from each strain was used as the template for DNA amplification. Primers originally developed by Lina *et al* were used to co-amplify *lukS-PV* and *lukF-PV*. [76]

Data Management

Data Cleaning

The original dataset was evaluated prior to any analysis using the following methods. To check the integrity of the dataset, the number of subjects in the dataset was required to match the number of individuals enrolled in the study. Also, each variable was checked for content, for missing values and for how they were coded; if missing values were coded with a number, they were set to "." using Stata, so the software recognized these values as being missing. Variables

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were checked for adequate coding. Dichotomous variables were coded as "0" for the reference group or "1" for the index group. Variables with more than two categories had the reference group coded as "0", and subsequent categories were coded as ordered integers, starting with "1". Indicator variables coded as "0" or "1" were created when the categorical variables were included in a statistical model, with the number of indicator variables equal to the number of categories in the original variable minus one. Any observation with an unusual value was compared with the original questionnaire or laboratory record, and corrected as necessary.

Handling Missing Data

All variables were checked to detect any missing data. When missing data elements were found, original questionnaires and laboratory records were reviewed to assess whether data were not entered into the dataset or were indeed missing. There was no missing data for any of the demographic and medical history variables. For analyses including colonization data, only those individuals who were swabbed during the specific month under analysis were included in the denominator. No assumptions were made about the colonization status of individuals who were not available during a specific period of time. Thus, when subjects were not present during a specific sampling period, their colonization status was set to missing and coded as "." using Stata.

Of the 377 enrolled subjects, 47.5% of them provided samples for at least half of the duration of the study, *i.e.* nine months or more. Of 6,786 potential observations, only 3,291 were obtained. This includes 89 subjects enrolled on the second year of study, of whom 68 were freshmen, and it also includes 42 seniors who left the study after the first year. Thus, the actual number of complete data points would have been 5,607, which means 58.7% of the colonization data was collected.

Assessing Correlations

Correlations between variables were assessed with Spearman's correlation coefficients. Covariates with a correlation higher than 70% were not included simultaneously in a multivariate model, to avoid instability of regression coefficients and inflation of standard errors. The correlation between type of sport (contact vs. noncontact) and sport was 87%, thus type of sport was the only sport variable included in statistical models. Besides, our interest was in assessing differences between contact and noncontact sports and not between individual sports. The next highest correlation was between gender and sport, but it did not reach our pre-specified cutoff.

Assessing Effect Measure Modification

Multiplicative interaction between covariates and the exposure of interest was assessed. Potential interactions were defined using *a priori* knowledge. Specifically, the interaction between type of sport (contact vs. noncontact) and gender was assessed for specific aim #2: assessing the association between type of sport and staphylococcal colonization. This was the least complex model, and had the most power to potentially detect an interaction. Interaction terms were generated as the multiplication of the exposure times the covariate of interest. Interactions were assessed using the likelihood ratio test to compare two nested regression models: the "full" model, containing the main effects and the interaction term, and the reduced model, containing only the main effects.

Assessing Confounding

Potential confounders to adjust for in multivariate models were chosen *a priori*, based on prior knowledge of previously reported confounders and known causal associations between covariates, exposure, and outcome. It has been reported that being male and being Caucasian are risk factors for staphylococcal colonization and infection.[2,5,110] Similarly, having previous

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staphylococcal infections or having a close contact who has had staphylococcal infections also increase the risk of having such infections and possibly colonization as well.[5] Having certain medical conditions such as diabetes, HIV, hemodialysis, obesity and skin diseases, also increases the risk of colonization and infection.[5]

Some of these risk factors could also be associated with the participation of an individual in contact or noncontact sports. Only men usually play some sports, such as football or baseball, and some of these sports could have a predominance of certain racial or ethnic groups. Also, if a subject tends to have recurrent staphylococcal infections or certain medical conditions, they would probably be more likely to participate in noncontact sports or not participate in sports at all.

Because of the potential causal association of the previously mentioned factors with both the type of sport (exposure) and staphylococcal colonization (outcome), gender, race/ethnicity, and several medical history variables were selected *a priori* to be included in multivariable models. Variables were included in the models as follows, using indicator variables when there were more than two categories: type of sport: contact or noncontact; gender: male or female; race: Caucasian, African-American or other; year: freshman, junior, sophomore, senior or trainer; history of staphylococcal infection: yes or no; previous staphylococcal infections in contacts: yes or no; previous surgeries: yes or no; any comorbidities: yes or no; antibiotics in the previous six months: yes or no.

Model Building Strategies

Explanatory Models

Explanatory models were used to assess associations between risk factors and the different colonization outcomes proposed in the specific aims. The goal of these models was to obtain the best estimate of the association between each exposure and colonization outcome, while adjusting for potential confounders and, when applicable, assessing potential effect measure modifiers. Exposures were modeled so that the group with the largest sample size was the reference category. Next, the crude measure of association between exposure and outcome was calculated. Later, assessment of correlations, effect measure modifiers and confounders were performed as mentioned earlier to build the final explanatory model.

Repeated Measures Analysis

This study involved obtaining repeated measures from the same individuals. Staphylococcal carriage information was generally obtained monthly for 18 months. Thus, an individual's carrier state was not defined by a single cross-sectional measurement, but rather by a series of measurements throughout the study period. Therefore, measurements obtained from a same individual were correlated, and cannot be considered independent for the purpose of statistical analyses. Further, most of the outcome variables in this study were nominal with three categories, such as no colonization, MSSA colonization or MRSA colonization at each time point. Several statistical methods can handle repeated measures of non-continuous outcomes, taking into consideration the correlation structure between measurements. One of these methods are generalized linear mixed models (GLMM). GLMM has some limitations, like making the assumption that the right side of the regression equation is linear, and that both the within- and between-subjects errors are normally distributed. Nonetheless, GLMM can handle nominal

outcome variables, and estimates both fixed-effect and random-effects. Consequently, GLMMs were used for most analyses in this study. The models were generalized logit models, with a glogit link function, and the distribution of the outcome variable was multinomial.

GLMMs are an extension of the more commonly known generalized linear models (GLMs). GLMs are a broad category of fixed-effects regression models for multiple types of outcome variables. These models include a linear predictor, a link function, and a variance term. The link function is a transformation of the expected value of the outcome variable that makes it equal to the linear predictor. This means that GLMs can be used even when there is not a linear association between the outcome variable and its predictors. However, fixed-effects regression models assume all observations are independent. In the present study, however, observations were not independent: each individual had multiple outcome measures, and thus, all measurements of colonization status within a single individual were correlated. In other words, this study involves multilevel data: the repeated observations (level 1) are nested within subjects (level 2). Thus, to account for the correlation between the observations, random-effects must be included in the models. Therefore, GLMMs are an extension of GLMs in that the former include random-effects in the linear predictor, in addition to the usual fixed-effects. Thus, GLMMs were an appropriate alternative for the current data, to account for the correlation of the observations as well as to handle a nominal outcome variable.

The following equations represent the multinomial mixed model used:

[Equation 1]

$$g(E[Y_{iz}|\mathbf{x_i}, \nu_i]) = \ln\left[\frac{P(Y_{iz} = 1|\mathbf{x_i}, \nu_i)}{P(Y_{iz} = 0|\mathbf{x_i}, \nu_i)}\right] = \text{logit}(P[Y_{iz} = 1|\mathbf{x_i}, \nu_i])$$

[Equation 2]

$$= \mathbf{x'}_{1i} \, \boldsymbol{\beta_1} + v_{1i}$$

where $v_{1i} \sim N(0, \tau_1)$

[Equation 3]

$$OR_{1k} = \exp(\beta_{1k})$$

and

[Equation 4]

$$g(E[Y_{iz}|\mathbf{x_i}, v_i]) = \ln\left[\frac{P(Y_{iz} = 2|\mathbf{x_i}, v_i)}{P(Y_{iz} = 0|\mathbf{x_i}, v_i)}\right] = \text{logit}(P[Y_{iz} = 2|\mathbf{x_i}, v_i])$$

[Equation 5]

$$= \mathbf{x'}_{2i} \, \boldsymbol{\beta_2} + v_{2i}$$

where $v_{2i} \sim N(0, \tau_2)$

[Equation 6]

$$OR_{2k} = \exp(\beta_{2k})$$

Y_{iz} = outcome for subject i at time z

$\boldsymbol{\beta_j}$ = vector of fixed effects coefficients for the jth logit

$\mathbf{x_i}$ = vector of predictor variables or regressors for subject i

v_i = random effects for subject i

τ_j = variance for the random effects for the jth logit

OR_{jk} = odds ratio for the jth logit and k^{th} predictor

Time-to-Event Analysis

To estimate the time to becoming colonized with *S. aureus* for non-colonized team members, a time-to-event analysis was performed. The time to becoming colonized for contact sports team members was compared to that of team members in noncontact sports. The main exposure was the sports team a student belongs to, categorized as contact or noncontact sport. The outcome of interest was time to nasal or oropharyngeal colonization with any *S. aureus*. Time to colonization was defined as the number of months from enrollment to the first detectable colonization with *S. aureus*. Athletes already colonized at enrollment were excluded from the analysis, so that only individuals who were not colonized at enrollment were included in the time-to-colonization analysis. Also, since monthly samples were not always collected on all athletes, the exact date when an athlete became colonized might not have been known; thus, some data were interval-censored. Further, those athletes who remained not colonized by the end of the study were right-censored. Consequently, given the presence of interval-censored data, the usual semi-parametric Cox proportional hazards model could not be used. Instead, a parametric survival model with a Weibull distribution was performed using R, to allow for interval censoring.[111] The same model was also performed limiting subjects to those who were freshmen, and thus new to their respective teams.

[Equation 7]

$$h_i(t) = \lambda_i \mathrm{p} t^{\mathrm{p}-1}$$

[Equation 8]

$$\lambda_i = \exp(\beta_0 + \beta_1 x_{i1} + \beta_2 x_{i2} + ... + \beta_K x_{iK})$$

[Equation 9]

$$HR_k = \exp(\beta_k)$$

p = shape parameter

$h_i(t)$ = hazard function for the ith subject

β_k = fixed effects coefficients for predictor k, k=1,…K

x_{ik} = predictor k for the ith subject

HR_k = hazard ratio for predictor k

Other Analyses

Logistic regression was used to assess the association between the types of MRSA colonizing

isolate and persistent or intermittent colonization. Multinomial logistic regression was used to

assess the association between contact sports and the carriage profile.

- Logistic Regression

[Equation 10]

$$g(E[Y_i|\mathbf{x_i}]) = \ln\left[\frac{P(Y_i = 1|\mathbf{x}_i)}{P(Y_i = 0|\mathbf{x}_i)}\right] = \text{logit}(P[Y_i|\mathbf{x_i}])$$

[Equation 11]

$$= \beta_0 + \beta_1 x_{i1} + \beta_2 x_{i2} + \dots + \beta_K x_{iK}$$

[Equation 12]

$$OR_k = \exp(\beta_k)$$

Y_i = outcome for subject i

β_k = fixed effect coefficient for predictor k, k=1,…K

$\mathbf{x_i}$ = vector of predictor variables or regressors for subject i

x_{ik} = predictor k for subject i

OR_k = odds ratio for variable k

- Multinomial Logistic Regression

$$g(E[Y_i|\mathbf{x_i}]) = \ln\left[\frac{P(Y_i = 1|\mathbf{x_i})}{P(Y_i = 0|\mathbf{x_i})}\right] = \text{logit}(P[Y_i = 1|\mathbf{x_i}])$$

$$= \beta_{10} + \beta_{11}x_{i1} + \beta_{12}x_{i2} + \ldots + \beta_{1K}x_{iK}$$

$$OR_{1k} = \exp(\beta_{1k})$$

and

$$g(E[Y_i|\mathbf{x_i}]) = \ln\left[\frac{P(Y_i = 2|\mathbf{x_i})}{P(Y_i = 0|\mathbf{x_i})}\right] = \text{logit}(P[Y_i = 2|\mathbf{x_i}])$$

$$= \beta_{20} + \beta_{21}x_{i1} + \beta_{22}x_{i2} + \ldots + \beta_{2K}x_{iK}$$

$$OR_{2k} = \exp(\beta_{2k})$$

Y_i = outcome for subject i

β_{jk} = fixed effect coefficient for the jth logit and k^{th} predictor

$\mathbf{x_i}$ = vector of predictor variables or regressors for subject i

x_{ik} = predictor k for subject i

OR_{jk} = odds ratio for the jth logit and k^{th} predictor

Specific Aim #1

To characterize the distribution of nasal and oropharyngeal colonization with *S. aureus* and community-associated methicillin-resistant *S. aureus* (CA-MRSA) in a prospective cohort of healthy collegiate student athletes over two academic years.

Sub-Aim #1.1: To estimate the seasonal prevalence of staphylococcal carriage both in the whole cohort and in the football team.

Sub-Aim #1.2: To estimate the time to colonization with *S. aureus* in new team members.

Sub-Aim #1.3: To assess whether prevalence of colonization differs by sub-types of CA-MRSA isolates and the virulence factors they possess.

Sub-Aim #1.4: To describe the effect of infection outbreaks on the prevalence of MRSA colonization in the football team. An outbreak was defined as "an increase in the rate of MRSA cases or a clustering of new cases due to the transmission of a single microbial strain." [14] Thus, we considered an outbreak whenever 2 or more infections occurred in members of the cohort due to indistinguishable isolates of MRSA in a period of time short enough that there might be a common source of exposure.

Rationale and Hypothesis

We hypothesized that the prevalence of both nasal and oropharyngeal colonization with *S. aureus* in general, and MRSA in particular, would fluctuate over time, depending on the timing within

each team's athletic season, frequency of infection outbreaks, and antibiotic use. We also hypothesized that sports with greater direct contact between athletes would have a higher prevalence of staphylococcal colonization, as compared to more individualized sports. Also, we hypothesized that new members of contact sports teams would become colonized in less time than those in noncontact sports. We also hypothesized that MRSA isolates that present a specific set of virulence factors, such as PVL and SCC*mec* type, would be differentially associated with colonization, and that prevalence of MRSA carriage would fluctuate around the time of an outbreak, and would likely decrease after decolonization or improved hygiene measures were taken.

Therefore, staphylococcal colonization was assessed in a prospective cohort of 377 healthy college athletes who were followed each month for two academic years. Detailed molecular data on the types of MRSA colonization isolates and the virulence factors possessed was analyzed. Defining the dynamics of *S. aureus* colonization over time will allow for improved surveillance of similar at-risk groups and provide new information about the longitudinal epidemiology of staphylococcal carriage.

Overview

From August 2008 to April 2010, monthly nasal and oropharyngeal swabs were obtained from participants from the Vanderbilt University Varsity Athletic Program. Additionally, a baseline questionnaire was collected, which assessed demographic and medical history variables that might be considered risk factors for staphylococcal colonization and infection. All isolates obtained were identified as either MSSA or MRSA. All MRSA isolates were further molecularly characterized, as previously described.

Data Analysis

Estimation of Prevalence of Nasal Carriage and Oropharyngeal Carriage

Carriage prevalence was defined as the number of positive cultures (numerator) within the number of subjects available for culture in the sample time period (denominator). This took into account participants who were unavailable for culture during the summer months when not all players reside on campus, nor take part in official team-related activities. Staphylococcal carriage prevalence was defined similarly as above, with the numerator being the number of cultures positive for any *S. aureus* – MSSA or MRSA. MSSA carriage prevalence and MRSA carriage prevalence had the number of cultures positive for MSSA or for MRSA in the numerator, respectively.

Estimation of seasonal staphylococcal carriage prevalence

The seasonal staphylococcal carriage prevalence was estimated as the average prevalence of staphylococcal colonization during each season –both for yearly seasons as for athletic seasons; the latter just for the football team. Yearly seasons were defined as summer (July-September), fall (October-December), winter (January-March) and spring (April-June). Athletic seasons for the football team were defined as pre-season (July-August), football season (September-November), post-season (December-February) and spring training (March-June). The seasonal prevalence was calculated first for the full cohort, and later for the football team, which has 125 subjects. Comparisons of seasons' prevalence were performed using a multinomial mixed model, to account for the correlation between the repeated measures within a same individual.

Model 1.1: full cohort

Exposure of interest: fall vs. winter vs. spring vs. summer.

Potential confounders: sport, gender, race/ethnicity, college year

Outcome: no staphylococcal carriage (reference) vs. MSSA carriage vs. MRSA carriage

Outcome counts: never carriers (**90**), ever MSSA (**112**), ever MRSA (**175**)

Maximum degrees of freedom: **9**

Table 1. Model 1.1: Predictors included in a multivariable regression model and their respective degrees of freedom

Predictor	Variable Name	d.f.	Original Levels
Year/Athletic Season	yearseason /athleticseason	3	Spring, Summer, Fall, Winter
Type of sport	contactsport	1	Noncontact sport, contact sport
Sex	sex	1	Female, male
Race	race3	2	Caucasian, African-American, Other
College year	year	3	Freshman, Sophomore, Junior, Senior
Total		10	

Model 1.2: football team

Exposure of interest: fall vs. winter vs. spring vs. summer, or football season vs. post-season vs. spring training vs. pre-season

Potential confounders: race/ethnicity, college year

Outcome: no staphylococcal carriage (reference) vs. MSSA carriage vs. MRSA carriage

Outcome counts: never carriers (**16**), ever MSSA (**33**), ever MRSA (**76**)

Maximum degrees of freedom: **1**

Table 2. Model 1.2: Predictors included in a multivariable regression model and their respective degrees of freedom

Predictor	Variable Name	d.f.	Original Levels
Year/Athletic Season	yearseason /athleticseason	3	Spring, Summer, Fall, Winter or football season vs. post-season vs. spring training vs. pre-season
Race	race3	2	Caucasian, African-American, Other
College year	year	3	Freshman, Sophomore, Junior, Senior
Total		8	

Estimation of time to becoming colonized with *S. aureus*

For those individuals not carrying *S. aureus* upon entry into the study, we assessed the time until

they became colonized with *S. aureus* –either MSSA or MRSA. The time of enrollment was t_0,

and the time at which the first *S. aureus* sample was obtained from them was t. For this, I performed a parametric survival analysis with a Weibull distribution, accounting for interval- and right-censored data, to obtain hazard ratios for becoming a carrier in contact sports' team members as compared to noncontact sports' team members. First, the analysis was performed including all cohort members, and later, including only freshmen.

Model 1.3: full cohort or freshmen

Exposures of interest: noncontact sports vs. contact sports.

Failure time: time from enrollment into the cohort without staphylococcal carriage to
   time of first positive nasal or oropharyngeal staphylococcal culture.

Potential confounders: gender, race/ethnicity, history of staphylococcal infections and
   season of enrollment

Outcome: time to SA carriage vs. no staphylococcal carriage (reference)

Outcome counts freshmen only: never carriers (**39**), ever SA (**40**)

Maximum degrees of freedom: **4**

Outcome counts full cohort: never carriers (**90**), ever SA (**96**)

Maximum degrees of freedom: **9**

Table 3. Model 1.3: Predictors included in a multivariable regression model and their respective degrees of freedom

Predictor	Variable Name	d.f.	Original Levels
Type of sport	contactsport	1	Noncontact sport, contact sport
Sex	sex	1	Female, male
Race	race3	2	Caucasian, African-American, Other
History of staphylococcal infection	staph_history	1	No, Yes
Season of enrollment	season_new	1	Summer, Fall
Total		6	

Prevalence of colonizing sub-types of MRSA and their virulence factors

In order to estimate the prevalence of various strain types, we estimated the relative frequency of nasal and oropharyngeal carriage of different MRSA types. To do this, first we estimated the relative frequency of different USA types, by including the number of isolates of a specific pulse-type in the numerator, and the total number of MRSA isolates obtained during the study in the denominator. Similarly, we also wanted to describe the relative frequency of the different SCC*mec* types, and the relative frequency of isolates containing the PVL-encoding genes.

Association between infections and the prevalence of MRSA colonization in the football team

To assess whether higher MRSA colonization prevalence resulted in subsequent infections, we described how the staphylococcal carriage prevalence changed around the time of the occurrence of a group of infections in the football team. To do this, we simply described the prevalence of colonization before, during, and after the time of the infections.

Power estimation

- Having 186 athletes, 95 contact sports athletes and 91 noncontact sports athletes, based on Stata's Cox proportional hazards power calculator, and assuming that the observed probability of becoming colonized with *S. aureus* (51.6%) was the true population rate, and the observed hazard ratio of 1.61, we have 65% power to detect a significant difference when comparing athletes in contact sports to athletes in noncontact sports. The Type I error probability associated with this test of this null hypothesis was 0.05.
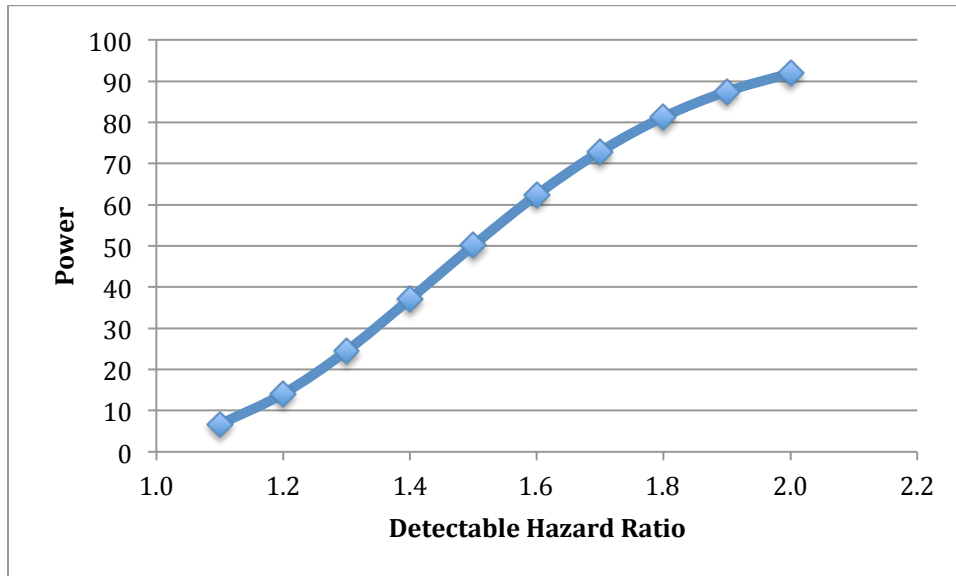
Figure 4. Power and detectable hazard ratio for becoming colonized with *S. aureus* in contact sports participants as compared to noncontact sports participants.

Potential Limitations/Alternative Approaches

This cohort study has an inherent limitation: the results might not be generalizable to a broader population. College athletes are a very particular population - they are typically healthier than the general population, are in a specific age range, and may have unique exposures based on living arrangements and sports participation. Nonetheless, studying such a cohort has several advantages, especially because it is a group at increased risk of staphylococcal colonization and infection. Thus, a more thorough understanding of the longitudinal dynamics and epidemiology of staphylococcal carriage in this specific cohort might shed light into the mechanisms and patterns of colonization in other populations.

A second potential limitation of this study is that only nasal and oropharyngeal cultures were used to determine colonization status. Given the nature of our cohort and the length of the study, cultures of the gastro-intestinal tract (through stool specimens) or of the perineum would very likely have resulted in decreased participation or compliance with visits. However, previous

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studies have shown the presence of SA in the axilla, throat and skin. [1,2,112-114] Further, the nares appear to be the most critical niche for the organism as evidenced by the disappearance of "extranasal" *S. aureus* colonization upon "intranasal" application of mupirocin. [115] The nose also appears to be the most clinically significant site of colonization as eradication from the nose often results in interruption of staphylococcal outbreaks by controlling transmission.[116] By selecting the oropharynx as a representative non-nasal culture site, we would be able to better define the precise ecology of CA-MRSA colonization while minimizing recruiting failures.

A potential limitation of time-to-event analysis is smaller sample size, which in turn limits the number of events, since only freshmen or those team members free of colonization at baseline were included in the analyses. However, the number of freshmen in the cohort was 149, almost 40% of the full cohort, and 110 of them were ever colonized with *S. aureus*. Another analysis was performed including all cohort members initially free of colonization. Even with the smaller sample size when looking only at freshmen, the results remained similar and statistically significant.

Specific Aim #2

To model the association between colonization with CA-MRSA in athletes and the type of sport they participate in, demographic characteristics, medical history and type of CA-MRSA strain, as compared to colonization with methicillin-susceptible *S. aureus* (MSSA) or no colonization, while adjusting for potential confounders and assessing effect measure modifiers.

Rationale and Hypothesis

We hypothesized that participating in contact sports would be associated with staphylococcal colonization, as compared to noncontact sports. Therefore, baseline information on demographics, medical history and team membership was used to explain subsequent staphylococcal colonization in a cohort of over 300 healthy college athletes who were followed each month for two academic years.

Overview

To assess risk factors for longitudinal staphylococcal colonization, monthly nasal and oropharyngeal swabs were obtained during an 18-month period from Vanderbilt University varsity athletes. Baseline information on demographics and medical history was collected through a questionnaire. All isolates obtained were identified as either MSSA or MRSA. All MRSA isolates were further molecularly analyzed, as previously described.

Data Analysis

Assessment of Risk Factors for Colonization

Univariate analysis of colonization and personal characteristics was performed using Pearson's chi-squared test or Fisher's exact test for categorical characteristics, as appropriate, and two-sample t-test or nonparametric Wilcoxon rank test for continuous variables, as appropriate. For assessing the impact of sport and particular risk factors on colonization status over time, the previously discussed GLMM analysis was utilized.

Model 2.1

Exposures of interest: noncontact sports vs. contact sports.

Potential confounders: gender, race/ethnicity, college year, history of staphylococcal

infection, history of staphylococcal infection in contacts, previous surgeries, co-

morbidities, and use of antibiotics in the previous 6 months

Effect measure modification: contact sport and gender

Outcome: no staphylococcal carriage (reference) vs. MSSA carriage vs. CA-MRSA

carriage.

Outcome counts: never carriers (**90**), ever MSSA (**112**), ever MRSA (**175**)

Maximum degrees of freedom: **9**

Table 4. Model 2.1: Predictors included in a multivariable regression model and their respective degrees of freedom

Predictor	Variable Name	d.f.	Original Levels
Type of sport	contactsport	1	Noncontact sport, contact sport
Sex	sex	1	Female, male
Race	race3	2	Caucasian, African-American, Other
College year	year	3	Freshman, Sophomore, Junior, Senior
History of staphylococcal infection	staph_history	1	No, yes
Previous staphylococcal infections in contacts	known_infection	1	No, yes
Medical conditions	medical_conditions_6	1	No, yes
Previous surgeries	surgeries2	1	No, yes
Antibiotics in previous 6 months	antibiotics	1	No, yes
Contact sport by gender interaction	contactsport*sex	1	No, yes
Total		13	

Sample size and power estimation

- With 224 contact sports athletes and 153 noncontact sports athletes, assuming an

  overall staphylococcal colonization rate among noncontact sports participants of

  20%, and a 35% among contact sports participants, we would be able to reject the

  null hypothesis that the colonization rate in both groups are equal with

  probability (power) 89.4%. The Type I error probability associated with this test

of this null hypothesis was 0.05. We used an uncorrected chi-squared statistic to evaluate this null hypothesis.
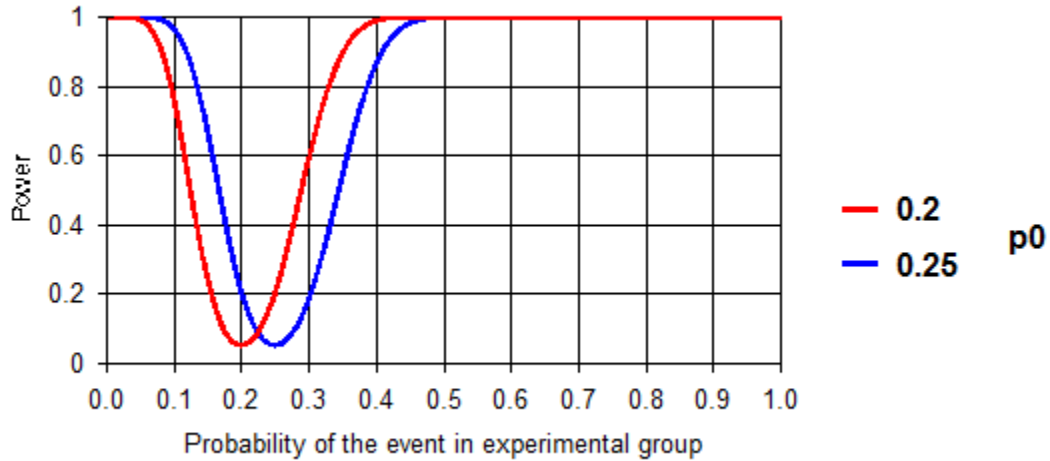
Figure 5. Power and detectable alternative proportion of colonization with *S. aureus* in contact sports participants

Possible Limitations/Alternative Approaches

A potential limitation of assessing staphylococcal colonization over an 18-month period is that not all individuals were sampled at all time points. Several individuals were absent during certain month's sampling. This would generate a significant missing data challenge. Using GLMM for assessing risk factors for colonization, however, does not eliminate an individual with incomplete data, but only discards the single missing observation. This modeling approach, consequently, makes the most use of the available data, without discarding subjects with some missing data points. By performing sensitivity analyses adjusting for the number of months subjects were swabbed and excluding subjects who contributed observations for only five months or less, the robustness of our results was assessed.

An alternative approach would be to conduct separate GEE models for several binary outcomes: no colonization vs. MSSA; no colonization vs. MRSA; and MSSA vs. MRSA. Although we

might have to adjust for multiple comparisons, we would be able to make all pairwise comparisons of interest between the three colonization statuses. Further, these analyses could also be performed both in aggregate and by body site colonized.

A third limitation was the categorization of all 14 athletic teams into just two categories, namely contact and noncontact sports. Though we would eventually like to evaluate the association between each specific team and staphylococcal colonization, we believe the broader categorization into contact and noncontact sports is more relevant. Further, we would lose many degrees of freedom if we decide to include the 14 teams in the GLMM analysis. If we find that contact sports are indeed significantly associated with higher colonization prevalence, we might later perform a GLMM analysis including all teams, to explore which of the teams was driving the association. Alternatively, we could run a GLMM model comparing football – the largest team – with the other contact sports and noncontact sports.

Also, the complexity of the model and the number of predictors to be included, combined with a potentially small number of colonization events, would require many degrees of freedom. Two alternative approaches could be taken. First, propensity scores could be used to summarize multiple predictor variables into a single propensity score value to adjust for in the multivariable model (Table 5). To do this, all covariates would first be used to build a logistic regression model that predicts the "propensity" or probability of being a contact sport athlete or a noncontact sport athlete. Later, this propensity score would be included as the only covariate, in addition to the exposure, in the GLMM analysis. A second potential approach would be to have a dichotomous outcome variable, which would allow for the use of a GEE model. This dichotomous variable would be defined as no colonization, or colonization with any *S. aureus*, either MSSA or MRSA, as specified in model 2.2. Propensity scores could be included as a covariate in this model as well.

Table 5. Predictors to be included in a multivariable regression model and their respective degrees of freedom, when using propensity scores

Predictor	Variable Name	d.f.	Original Levels
Type of sport	contactsport	1	Noncontact sport, contact sport
Sex	sex	1	Female, male
Interaction of sport and sex		1	
Propensity score		1	
Total		**4**	

Model 2.2

Exposures of interest: noncontact sports vs. contact sports.

Potential confounders: gender, race/ethnicity, college year, history of staphylococcal infection, history of staphylococcal infection in contacts, use of antibiotics in the previous 6 months

Outcome: no staphylococcal carriage (reference) vs. SA carriage.

Outcome counts: never carriers (**90**), ever SA (**287**)

Maximum degrees of freedom: **9**

Specific Aim #3

To model the association between persistent staphylococcal colonization and type of sport, site of colonization and specific staphylococcal virulence factors, as compared to intermittent colonization or no colonization with *S. aureus*, while adjusting for potential confounders.

Sub-Aim #3.1: To model the association between persistent staphylococcal colonization in athletes and the type of sport they participate in, demographic and medical history variables, as compared to intermittent colonization or no colonization with *S. aureus*, while adjusting for potential confounders.

Sub-Aim #3.2: To model the association between persistent staphylococcal colonization in athletes and site of colonization, while adjusting for potential confounders.

Sub-Aim #3.3: To model the association between persistent staphylococcal colonization and the molecular characteristics present in MRSA isolates obtained from collegiate athletes.

Rationale and Hypothesis

We hypothesized that people who are persistently colonized with *S. aureus* differ from those who are only intermittently colonized or not colonized, either in personal characteristics or in the type of isolate they are colonized with. Persistent carriers were defined as those individuals who were colonized during ≥80% of the months they were available for sampling; non-carriers were those who were never colonized, and intermittent carriers were those colonized once or more, but less than 80% of the time. We hypothesized that contact sports participants would be more likely to be persistent staphylococcal carriers, as well as those athletes colonized with *S. aureus* in both nose and oropharynx. We also hypothesized that MRSA isolates that present a specific set of molecular characteristics would be differentially able to cause persistent rather than intermittent colonization. To test these hypotheses, we assessed whether an association exists between demographics, medical history, team membership, site of colonization and type of colonizing isolate, and whether athletes enrolled in the cohort were persistently colonized or not.

Overview

Monthly nasal and oropharyngeal swabs were obtained during an 18-month period from Vanderbilt University varsity athletes. Those who were staphylococcal carriers during ≥80% of the time at which they were sampled were categorized as persistent carriers. Those who were never colonized were categorized as non-carriers, and those colonized at least once, but <80% of the time they were swabbed, were classified as intermittent carriers. Baseline information on demographics and medical history was collected through a questionnaire. All isolates obtained

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were identified as either MSSA or MRSA. All MRSA isolates were further molecularly analyzed, as previously described.

Data Analysis

Estimating the Association Between Persistent Staphylococcal Carriage and Sport

To assess whether athletes who participate in contact sports are more likely to be persistent staphylococcal carriers than those who participate in noncontact sports, a multinomial logistic regression analysis was performed. To compare the three carrier profiles, contrasts were used to obtain odds ratios for being persistent, intermittent, or non-carriers among contact sports participants, when compared to noncontact sports participants.

Model 3.1

Exposures of interest: noncontact sports vs. contact sports.

Potential confounders: gender, race/ethnicity, college year

Outcome: no staphylococcal carriage (reference) vs. intermittent SA carriage vs. persistent SA carriage.

Outcome counts: no staphylococcal carriage (**90**), intermittent SA carriage (**200**), persistent SA carriage (**87**)

Maximum degrees of freedom: **8**

Table 6. Model 3.1: Predictors included in a multivariable regression model and their respective degrees of freedom

Predictor	Variable Name	d.f.	Original Levels
Type of sport	contactsport	1	Noncontact sport, contact sport
Sex	sex	1	Female, male
Race	race3	2	Caucasian, African-American, Other
College year	year	3	Freshman, Sophomore, Junior, Senior
Total		7	

Estimating the Association Between Persistent Staphylococcal Carriage and Colonization Site

To assess whether those athletes who were persistently colonized were more likely to be colonized over time in the anterior nares and oropharynx than those who were colonized at only one site, a multinomial mixed regression analysis (GLMM) was performed. Those who were never colonized were excluded from this analysis.

Model 3.2

Exposures of interest: intermittent SA carriage vs. persistent SA carriage

Potential confounders: sport, gender, race/ethnicity, college year

Outcome: nasal SA carriage only (reference) vs. oropharyngeal SA carriage only vs. SA carriage in both sites

Outcome counts: intermittent SA carriage (**200**), persistent SA carriage (**87**)

Maximum degrees of freedom: **8**

Table 7. Model 3.2: Predictors included in a multivariable regression model and their respective degrees of freedom

Predictor	Variable Name	d.f.	Original Levels
Persistent or intermittent carrier	persistent_intermittent	1	Persistent, intermittent
Type of sport	contactsport	1	Noncontact sport, contact sport
Sex	sex	1	Female, male
Race	race3	2	Caucasian, African-American, Other
College year	year	3	Freshman, Sophomore, Junior, Senior
Total		9	

Estimating the Association Between Persistent Staphylococcal Carriage and Type of Colonizing MRSA Isolate

To assess whether those athletes colonized with a specific type of MRSA isolate were more likely to be persistent staphylococcal carriers than those who were colonized with other types, a logistic

regression analysis was performed. Those subjects who were never colonized during the study period were excluded from this analysis.

Model 3.3

Exposures of interest: other pulse types vs. USA300, PVL negative vs. PVL positive, SCCmec types I, II, III or V vs. SCCmec type IV

Potential confounders: sport, gender, race/ethnicity

Outcome: intermittent MRSA carriage (reference) vs. persistent MRSA carriage

Outcome counts: intermittent SA carriage (**200**), persistent SA carriage (**87**)

Maximum degrees of freedom: **8**

Table 8. Model 3.3: Predictors included in a multivariable regression model and their respective degrees of freedom

Predictor	Variable Name	d.f.	Original Levels
Pulse type [a]	usa300	1	Other pulse types, USA 300
PVL positive [a]	pvl	1	PVL absent, PVL present
SCC*mec* type [a]	sccmec_iv	1	SCC*mec* I, II, III or V, SCC*mec* IV
Type of sport	contactsport	1	Noncontact sport, contact sport
Sex	sex	1	Female, male
Race	race3	2	Caucasian, African-American, Other
Total		7	

[a]: One of these predictors in the model at a time

Sample size and power estimation

- With 224 contact sports athletes and 153 noncontact sports athletes, assuming a persistent staphylococcal colonization rate among noncontact sports participants of 12%, and a 20% among contact sports participants, we would be able to reject the null hypothesis that the colonization rate in both groups are equal with probability (power) 53.3%. The Type I error probability associated with this test of this null hypothesis was 0.05. We used an uncorrected chi-squared statistic to evaluate this null hypothesis.
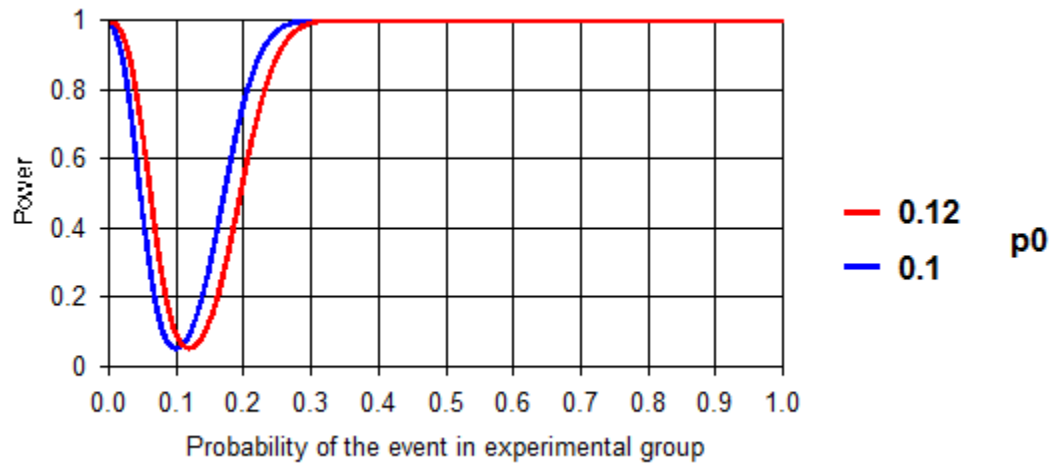
Figure 6. Power and detectable alternative proportion of persistent colonization with *S. aureus* in contact sports participants

Potential Limitations/Alternative Approaches

One of the limitations of the analyses proposed for aim #3 is the categorization of the 14 athletic

teams into just two categories: contact and noncontact sports. Though we would eventually like to

evaluate the association between each specific team and the different staphylococcal colonization

patterns, we believe the broader categorization into contact and noncontact sports is more

relevant. Further, we would lose many degrees of freedom if we decide to include the 14 teams in

the analyses. If we find that contact sports are indeed significantly associated with a persistent

carrier pattern, we might later perform a multinomial logistic regression analysis including all

teams, to explore which of the teams was driving the association. Alternatively, we could fit a

multinomial logistic regression model comparing football – the largest team – with the other

contact sports and noncontact sports.

Planned Papers

A first paper will be primarily descriptive. It will be based on the first specific aim and its sub-aims, and thus, will describe the dynamics of longitudinal prevalence of staphylococcal colonization in a cohort of healthy collegiate student athletes. Initially, this paper will include a detailed description of study methods, involving selection and recruitment of the cohort. Laboratory methods for culturing isolates will also be included in the paper, as well as molecular methods for detecting virulence factors and other determinants of CA-MRSA. Additionally, the paper will include a description of the enrolled cohort, including sports teams, demographics and medical history of the athletes. This information will be presented in univariate analysis by team. Furthermore, the estimates of prevalence of colonization with *S. aureus* and CA-MRSA will be shown. The temporal trends in colonization, as well as the analysis on time-to-becoming colonized will be presented, too, as well as a description of the colonization prevalence around the time of staphylococcal infections in the cohort.

A second paper will be based on the second specific aim. Thus, the focus of the paper will be to assess the association between several predictor variables and staphylococcal carriage in this cohort of student athletes. Since the more detailed description of the study was done in the first paper, this second paper will only give a brief summary of study methods, and will refer to the first paper for more detailed information. A thorough description will be done for the statistical methods for modeling the risk of staphylococcal colonization in this cohort. Initially, the prevalence estimates of colonization with MSSA and MRSA will be presented for each athletic team over time. Subsequently, staphylococcal carriage prevalence will be compared in contact sports participants and noncontact sports participants, to determine the unadjusted odds ratio of colonization and the adjusted odds ratio of colonization using a generalized linear mixed model. The model will be adjusted for potential confounders, and effect measure modifiers will be

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assessed. Comparisons will be made both in aggregate (either oropharyngeal or nasal colonization) and by individual carriage site.

A third paper will be based on the third specific aim. The prevalence of persistent and intermittent carriers will be described, as proposed in specific aim #3. Later I will describe the association between sport, colonization type, and type of MRSA isolate and the three different colonization profiles, estimated using multinomial logistic regression.

Alternative Approaches

An alternative for the planned papers would be to join specific aims 2 and 3 into one paper. Another alternative would be to include a methodological paper, conducting a sensitivity analysis to compare the GLMM approach with a GEE approach for those outcome variables that are binary. Both methods would be compared in terms of assumptions, precision, results and implications.

CHAPTER III

RESULTS: STAPHYLOCOCCAL COLONIZATION PREVALENCE

IN STUDENT ATHLETES

Specific Aim #1

To characterize the distribution of nasal and oropharyngeal colonization with *S. aureus* and community-associated methicillin-resistant *S. aureus* (CA-MRSA) in a prospective cohort of healthy collegiate student athletes over two academic years.

Overall staphylococcal colonization

To assess the prevalence of staphylococcal colonization over time in a cohort of healthy athletes, nasal and oropharyngeal swabs were collected from 377 athletes and trainers, over 18 sampling periods. Of these athletes, 224 were categorized as contact sports players (football, basketball, soccer, lacrosse), and 153 as noncontact sports players (cross country, tennis, golf, bowling, swimming, baseball, and trainers; Table 9). Most of the athletes were males (57.29%), Caucasians (74.27%), and were in their freshmen year (39.52%). The largest team was football, with 125 athletes enrolled, followed by baseball with 36 and lacrosse with 34.

Table 9. Frequency of sport affiliation, demographic factors and medical history for a cohort of 377 college student athletes at Vanderbilt University, by overall colonization status

Variable	Total Cohort N=377 N (%)	Never Colonized N=90 N (%)	Ever Colonized with *S. aureus* N=287 N (%)	P
Sport		90 (23.87)	287 (76.13)	0.001[P]
M. Football	125 (33.16)	16 (17.78)	109 (37.98)	
W. Lacrosse	34 (9.02)	3 (3.33)	31 (10.80)	
M. Basketball	17 (4.51)	4 (4.44)	13 (4.53)	
W. Basketball	19 (5.04)	5 (5.56)	14 (4.88)	
W. Soccer	29 (7.69)	7 (7.78)	22 (7.67)	
M. Baseball	36 (9.55)	9 (10.00)	27 (9.41)	
M. Tennis	12 (3.18)	5 (5.56)	7 (2.44)	
W. Tennis	10 (2.65)	2 (2.22)	8 (2.79)	
M. Cross Country	10 (2.65)	2 (2.22)	8 (2.79)	
W. Cross Country	14 (3.71)	5 (5.56)	9 (3.14)	
W. Track and Field	11 (2.92)	3 (3.33)	8 (2.79)	
W. Bowling	15 (3.98)	9 (10.00)	6 (2.09)	
M. Golf	10 (2.65)	4 (4.44)	6 (2.09)	
W. Swimming	23 (6.10)	11 (12.22)	12 (4.18)	
Trainers	12 (3.18)	5 (5.56)	7 (2.44)	
Categorized Sports				< 0.001[P]
Noncontact	153 (40.58)	55 (61.11)	98 (34.15)	
Contact	224 (59.42)	35 (38.89)	189 (65.85)	
Gender				0.019[P]
Female	161 (42.71)	48 (53.33)	113 (39.37)	
Male	216 (57.29)	42 (46.67)	174 (60.63)	
Race/Ethnicity				0.973[F]
Caucasian	280 (74.27)	68 (75.56)	212 (73.87)	
African-American	80 (21.22)	18 (20.00)	62 (21.60)	
Other	17 (4.51)	4 (4.44)	13 (4.53)	
College Year				0.294[F]
Freshman	149 (39.52)	39 (43.33)	110 (38.33)	
Sophomore	87 (23.08)	16 (17.78)	71 (24.74)	
Junior	70 (18.57)	14 (15.56)	56 (19.51)	
Senior	59 (15.65)	16 (17.78)	43 (14.98)	
Trainers	12 (3.18)	5 (5.56)	7 (2.44)	
History of SA infection				0.310[F]
No	355 (94.16)	87 (96.67)	268 (93.38)	
Yes	22 (5.84)	3 (3.33)	19 (6.62)	

Table 9, continued…

Variable	Total Cohort	Never Colonized	Ever Colonized with *S. aureus*	P
	N=377	N=90	N=287	
	N (%)	N (%)	N (%)	
SA infection in contacts				0.732[P]
No	261 (69.23)	61 (67.78)	200 (69.69)	
Yes	116 (30.77)	29 (32.22)	87 (30.31)	
Previous surgeries				0.023[P]
No	275 (72.94)	74 (82.22)	201 (70.03)	
Yes	102 (27.06)	16 (17.78)	86 (29.97)	
Comorbidities				0.537[F]
No	362 (96.02)	88 (97.78)	274 (95.47)	
Yes	15 (3.98)	2 (2.22)	13 (4.53)	
Antibiotics in Previous 6 Months				0.845[P]
No	329 (87.27)	78 (86.67)	251 (87.46)	
Yes	48 (12.73)	12 (13.33)	36 (12.54)	
MRSA Colonization				< 0.001[F]
Never Colonized	202 (53.58)	90 (100.00)	112 (39.02)	
Ever MRSA	175 (46.42)	0 (0.00)	175 (60.98)	

[P]: Pearson's Chi-squared
[F]: Fisher's exact test

Overall, 3,291 longitudinal observations (3,291 nasal swabs and 3,291 oropharyngeal swabs) were collected from 377 athletes over the course of the study (Table 10). Football contributed 1,362 observations, followed by the lacrosse team with 357 observations, soccer with 275 and men's basketball with 181. Though the baseball team was the second largest in number of athletes, it only contributed 149 observations.

Of the 377 athletes, 90 (23.87%) were never colonized with SA, while 287 (76.13%) were colonized with SA and 175 (46.4%) with MRSA at some point during the study (Table 9**Error! Reference source not found.**). SA was detected in 1,433 (43.54%) of the 3,291 samples; MSSA was isolated from 890 (27.04%) samples and MRSA from 543 samples (16.50%**Error! Reference source not found.**).

Table 10. Number of individuals swabbed and colonized by month

		Swabbed (denominator)[a] n (%) n = 3,291	Colonized with SA[b] n (%) 1,433 (43.54)	Colonized with MSSA[b] n (%) 890 (27.04)	Colonized with MRSA[b] n (%) 543 (16.50)
Month 1	August 2008	100 (26.53)	62 (62.00)	42 (42.00)	20 (20.00)
Month 2	September 2008	140 (37.14)	77 (55.00)	54 (38.57)	23 (16.43)
Month 3	October 2008	202 (53.58)	90 (44.55)	64 (31.68)	26 (12.87)
Month 4	November 2008	262 (69.50)	110 (41.98)	88 (33.59)	22 (8.40)
Month 5	December 2008	232 (61.54)	89 (38.36)	61 (26.29)	28 (12.07)
Month6	January 2009	232 (61.54)	95 (40.95)	63 (27.16)	32 (13.79)
Month 7	February 2009	241 (63.93)	112 (46.47)	69 (28.63)	43 (17.84)
Month 8	March 2009	247 (65.52)	109 (44.13)	60 (24.29)	49 (19.84)
Month 9	April 2009	87 (23.08)	35 (40.23)	18 (20.69)	17 (19.54)
Month 10	May 2009				
Month 11	June 2009				
Month 12	July 2009	86 (22.81)	49 (56.98)	24 (27.91)	25 (29.07)
Month 13	August 2009	103 (27.32)	49 (47.57)	19 (18.45)	30 (29.13)
Month 14	September 2009	287 (76.13)	137 (47.74)	71 (24.74)	66 (23.00)
Month 15	October 2009	245 (64.99)	84 (34.29)	60 (24.49)	24 (9.80)
Month 16	November 2009	143 (37.93)	53 (37.06)	35 (24.48)	18 (12.59)
Month 17	December 2009				
Month 18	January 2010	212 (56.23)	98 (46.23)	60 (28.30)	38 (17.92)
Month 19	February 2010	183 (48.54)	76 (41.53)	42 (22.95)	34 (18.58)
Month 20	March 2010	208 (55.17)	77 (37.02)	43 (20.67)	34 (16.35)
Month 21	April 2010	81 (21.49)	31 (38.27)	17 (20.99)	14 (17.28)

[a]: Percentages are from the total number of participants (377)
[b]: Percentages are from the number of participants swabbed each month

The prevalence of staphylococcal carriage was dynamic over time (Figure 7). The highest overall colonization prevalence was in August 2008, when 62% of those swabbed were colonized with SA; the lowest prevalence was 34% in October 2009. The highest MRSA prevalence was 29%, during the summer of the second year. When stratifying by type of sport, staphylococcal colonization prevalence seemed higher in those athletes who participate in contact sports, where between 32% and 62% were colonized, while prevalence in noncontact sports athletes ranged between 18% and 53% (Figure 8). In the football team alone, SA prevalence ranged from 23% to 62% (Figure 9).
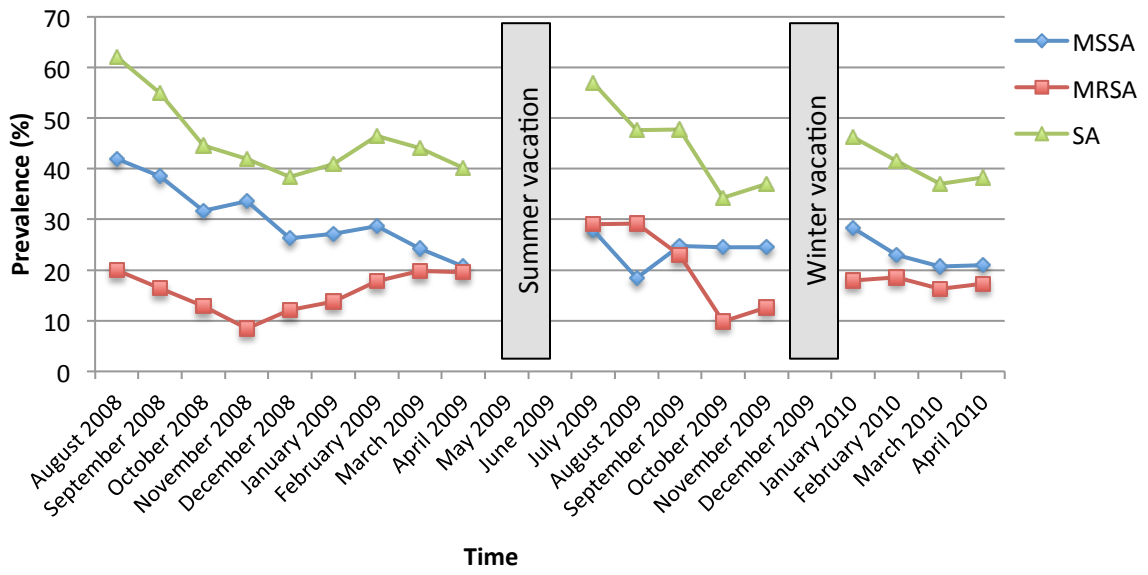
Figure 7. Prevalence of staphylococcal colonization in a cohort of healthy college student athletes over two academic years
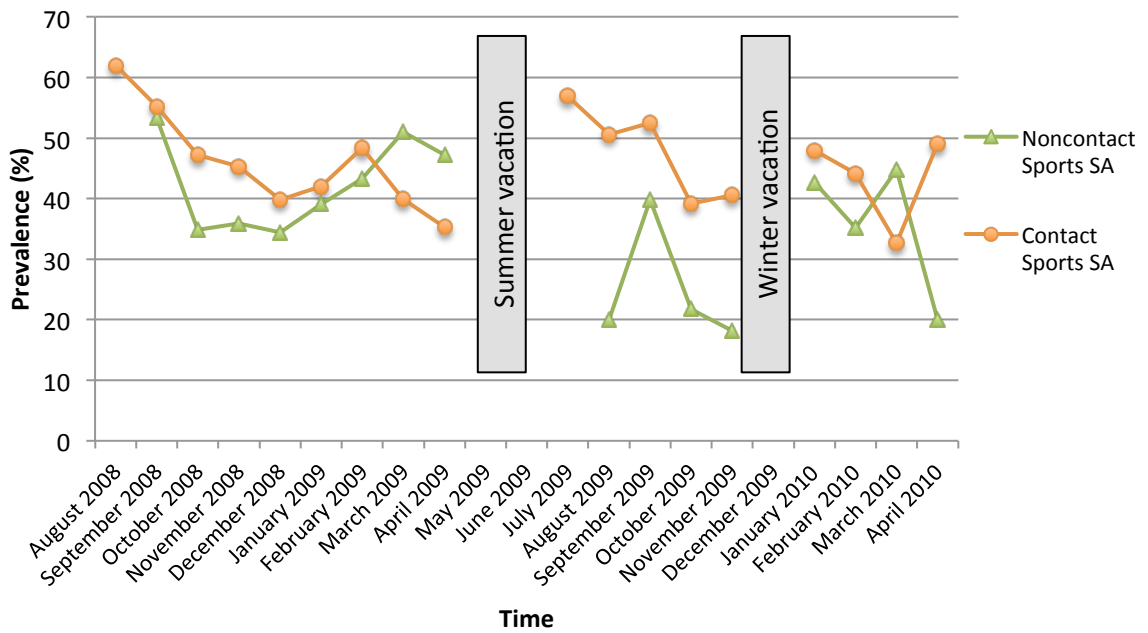
Figure 8. Prevalence of staphylococcal colonization in contact vs. noncontact sports college athletes over two academic years

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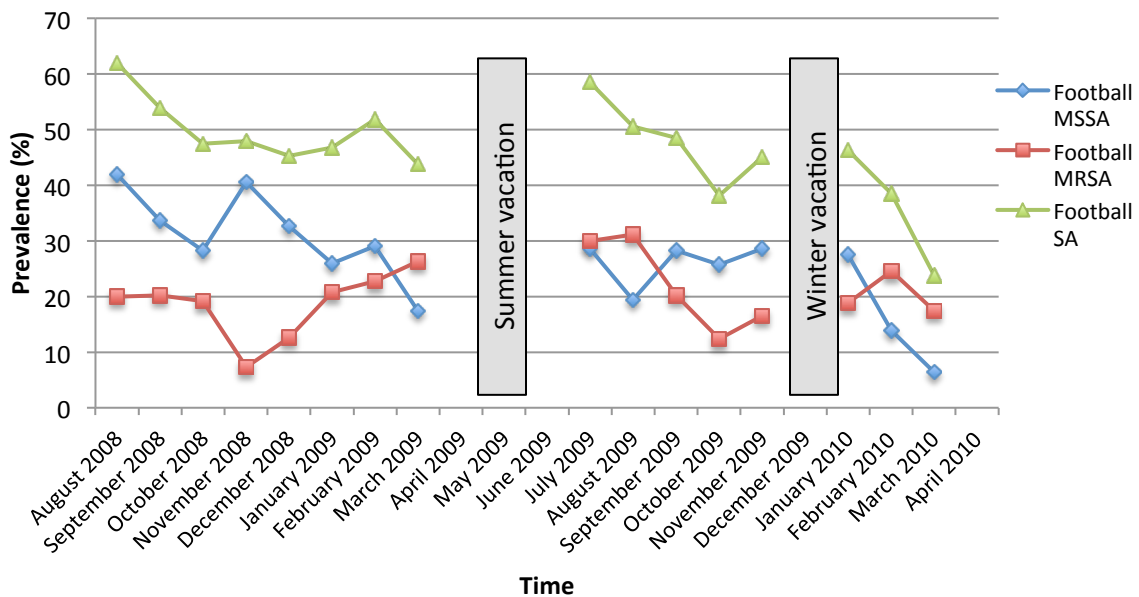
Figure 9. Prevalence of staphylococcal colonization in the football team over two academic years

Nasal and oropharyngeal staphylococcal colonization

To assess whether nasal and oropharyngeal staphylococcal colonization trends were similar over time in a cohort of healthy athletes, staphylococcal colonization prevalence was examined separately by colonization site, over 18 sampling periods. Nasal carriage of MSSA and MRSA was higher than oropharyngeal carriage, though the difference seemed greater for MRSA carriage (Figure 10, Figure 11). The highest prevalence of oropharyngeal colonization was 47%, while for nasal colonization the highest prevalence was 41%.
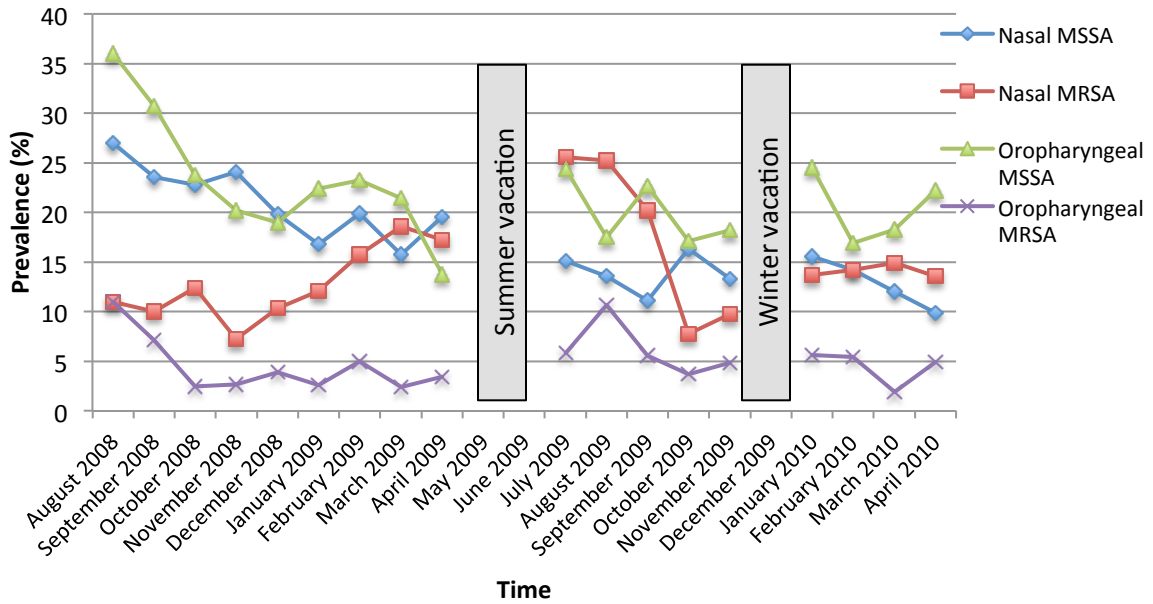
Figure 10. Prevalence of nasal vs. oropharyngeal staphylococcal colonization in a cohort of healthy college student athletes over two academic years
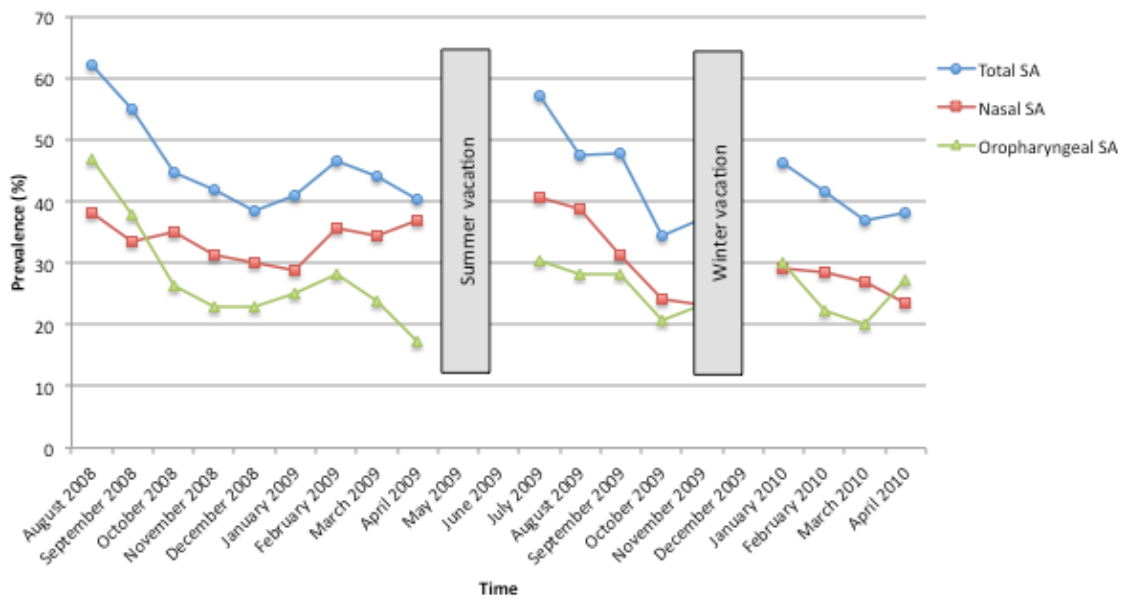
Figure 11. Prevalence of nasal vs. oropharyngeal SA colonization in a cohort of healthy college student athletes over two academic years

To increase the sensitivity in detecting colonized individuals, axillary swabs were also obtained, but only during the first 5 months of the study. A total of 926 armpit swabs from 287 individuals were obtained; 91 of these swabs (9.83%) from 54 individuals (18.82%) had either MSSA (74) or MRSA (17). Five individuals of 54 (9.26%), three of them from contact sports teams, had 6 armpit swabs with SA, which was not detected in either nasal or oropharyngeal swabs. Thus, axillary samples were not collected further, nor were they considered in any analysis.

Of 3,291 non-missing data points during the duration of the study, we missed 2,365 axillary swabs (3,291 minus 926); assuming 9.83% of these missed swabs had SA, 232.5 positive axillary samples would have been missed. Of the total number of subjects (377), 18.82%, or 70.95 subjects would have had axillar SA. Of these, 9.26% (or 1.74% from the full cohort of 377) would have been colonized only in the axilla and thus potentially misclassified as never colonized; as such, 6.57 individuals would have been missed as colonized. If 1.74% of all individuals swabbed were only colonized in armpit, and never colonized in either nose or throat, and thus misclassified as non-carriers of SA, approximately 7 subjects of 377, less than 2%, would have been misclassified.

To evaluate whether those who were colonized at a specific body site on a given month were more likely to continue to be colonized in the same site the next month, a multinomial mixed model with random intercepts was performed, comparing previous months' colonization sites with current months' colonization. Those athletes who had both nasal and oropharyngeal staphylococcal colonization on the previous month, were 7-fold as likely to have nasal and oropharyngeal colonization again, twice as likely to have only nasal colonization and 4-fold as likely to have oropharyngeal colonization only than no colonization on the current month, as compared to those with no colonization on the previous month (Table 11). Similarly, those with previous nasal colonization alone and those with oropharyngeal colonization alone were more

likely to have nasal, oropharyngeal or nasal and oropharyngeal colonization, as opposed to not

being colonized, compared to those with no colonization on the previous month. Sensitivity

analyses are presented on Table 25, Appendix B.

Table 11. Odds ratios for current staphylococcal carriage as a function of previous staphylococcal carriage in 377 subjects with 2,405 observations, based on multinomial mixed models with random intercept

Colonization on Previous Month	Crude Model[a]		Adjusted Model[a,b]	
	OR	95% CI	OR	95% CI
Not colonized on previous month	1.00	---	1.00	---
Nasal and oropharyngeal on previous month				
Nasal and oropharyngeal	7.10	4.26, 11.82	6.97	4.17, 11.66
Nasal only	1.99	1.19, 3.35	2.00	1.18, 3.37
Oropharyngeal only	4.24	2.83, 6.36	4.28	2.85, 6.43
Nasal only on previous month				
Nasal and oropharyngeal	27.30	17.28, 43.13	26.04	16.45, 41.21
Nasal only	20.60	13.97, 30.38	20.18	13.63, 29.88
Oropharyngeal only	1.80	1.05, 3.08	1.77	1.03, 3.04
Oropharyngeal only on previous month				
Nasal and oropharyngeal	68.58	39.55, 118.93	63.55	36.52, 110.61
Nasal only	32.38	20.66, 50.76	31.45	19.98, 49.52
Oropharyngeal only	7.56	4.51, 12.65	7.64	4.58, 12.76
Month (time)				
Nasal and oropharyngeal	0.98	0.95, 1.01	0.98	0.95, 1.01
Nasal only	0.99	0.96, 1.01	0.99	0.96, 1.01
Oropharyngeal only	1.02	1.00, 1.05	1.03	1.01, 1.06

[a]: To be included in the model, both current and previous month variables could not be missing: from 3,291 observations, only 2,405 were included in the analysis.
[b]: Adjusted for contact sport (contact vs. noncontact), gender, race, college year

Table 12. Number of positive samples (nasal or oropharyngeal) by month and type of sport

		Noncontact sports (swabs n=950)			Contact sports (swabs n=2,341)			Football (swabs n=1,362)		
		Total SA **n (%)** **n = 365**	**MSSA** **n (%)** **n = 232**	**MRSA** **n (%)** **n = 133**	**Total SA** **n (%)** **n = 1,068**	**MSSA** **n (%)** **n = 658**	**MRSA** **n (%)** **n = 410**	**Total SA** **n (%)** **n = 644**	**MSSA** **n (%)** **n = 376**	**MRSA** **n (%)** **n = 268**
Month 1	August 2008				62 (62.0)	42 (42.0)	20 (20.0)	62 (62.0)	42 (42.0)	20 (20.0)
Month 2	September 2008	8 (53.3	8 (53.3)	0 (0.0)	69 (55.2)	46 (36.8)	23 (18.4)	48 (53.9)	30 (33.7)	18 (20.2)
Month 3	October 2008	15 (34.9)	13 (30.2)	2 (4.6)	75 (47.2)	51 (32.1)	24 (15.1)	47 (47.5)	28 (28.3)	19 (19.2)
Month 4	November 2008	33 (35.9)	24 (26.1)	9 (9.8)	77 (45.3)	64 (37.6)	13 (7.6)	46 (47.9)	39 (40.6)	7 (7.3)
Month 5	December 2008	21 (34.4)	14 (22.9)	7 (11.5)	68 (39.8)	47 (27.5)	21 (12.3)	43 (45.3)	31 (32.6)	12 (12.6)
Month6	January 2009	32 (39.0)	21 (25.6)	11 (13.4)	63 (42.0)	42 (28.0)	21 (14.0)	36 (46.7)	20 (26.0)	16 (20.8)
Month 7	February 2009	39 (43.3)	26 (28.9)	13 (14.4)	73 (48.3)	43 (28.5)	30 (19.9)	41 (51.9)	23 (29.1)	18 (22.8)
Month 8	March 2009	48 (51.1)	32 (34.0)	16 (17.0)	61 (39.9)	28 (18.3)	33 (21.6)	35 (43.7)	14 (17.5)	21 (26.2)
Month 9	April 2009	17 (47.2)	9 (25.0)	8 (22.2)	18 (35.3)	9 (17.6)	9 (17.6)			
Month 10	May 2009									
Month 11	June 2009									
Month 12	July 2009				49 (57.0)	24 (27.9)	25 (29.1)	41 (58.6)	20 (28.6)	21 (30.0)
Month 13	August 2009	2 (20.0)	1 (10.0)	1 (10.0)	47 (50.5)	18 (19.3)	29 (31.2)	47 (50.5)	18 (19.3)	29 (31.2)
Month 14	September 2009	43 (39.8)	18 (16.7)	25 (23.1)	94 (52.5)	53 (29.6)	41 (22.9)	48 (48.5)	28 (28.3)	20 (20.2)
Month 15	October 2009	15 (21.7)	10 (14.5)	5 (7.2)	69 (39.2)	50 (28.4)	19 (10.8)	37 (38.1)	25 (25.8)	12 (12.4)
Month 16	November 2009	4 (18.2)	4 (18.2)	0 (0.0)	49 (40.5)	31 (25.6)	18 (14.9)	41 (45.0)	26 (28.6)	15 (16.5)
Month 17	December 2009									
Month 18	January 2010	29 (42.6)	16 (23.5)	13 (19.1)	69 (47.9)	44 (30.6)	25 (17.4)	32 (46.4)	19 (27.5)	13 (18.8)
Month 19	February 2010	19 (35.2)	14 (25.9)	5 (9.3)	57 (44.2)	28 (21.7)	29 (22.5)	25 (38.5)	9 (13.8)	16 (24.6)
Month 20	March 2010	34 (44.7)	19 (25.0)	15 (19.7)	43 (32.6)	24 (18.2)	19 (14.4)	15 (23.8)	4 (6.3)	11 (17.5)
Month 21	April 2010	6 (20.0)	3 (10.0)	3 (10.0)	25 (49.0)	14 (27.4)	11 (21.6)			

Estimation of seasonal staphylococcal carriage prevalence

To assess whether colonization with MSSA and MRSA differed across seasons, we compared the odds of colonization during each season for the full cohort. Based on a multinomial mixed model with random intercepts using all 3,291 observations from 377 subjects, and adjusting for gender, type of sport, race, and college year, the odds of being colonized with both MSSA and MRSA in the summer were significantly higher than the odds of being colonized in the winter (Table 13). Also, the odds of being colonized with MRSA in the fall were significantly lower than the odds of having MRSA in the winter.

Table 13. Odds ratios for crude and adjusted multinomial mixed models with random intercept to assess the association between seasons and staphylococcal colonization

	Full Cohort				Football team			
	Crude Model		Adjusted Model[a]		Crude Model		Adjusted Model[b]	
	OR	95% CI	OR	95% CI	OR	95% CI	OR	95% CI
Calendar Season								
Winter	1.00	---	1.00	---	1.00	---	1.00	---
Spring								
MRSA	1.49	0.84, 2.63	1.52	0.86, 2.70				
MSSA	0.84	0.51, 1.39	0.85	0.52, 1.41				
Summer								
MRSA	1.78	1.29, 2.46	1.70	1.23, 2.35	1.53	0.99, 2.36	1.52	0.99, 2.35
MSSA	1.41	1.07, 1.85	1.38	1.05, 1.82	1.70	1.15, 2.51	1.69	1.15, 2.50
Fall								
MRSA	0.46	0.33, 0.63	0.45	0.32, 0.62	0.49	0.31, 0.77	0.48	0.30, 0.76
MSSA	0.90	0,71, 1.14	0.89	0.70, 1.13	1.32	0.91, 1.93	1.30	0.89, 1.90
Athletic Season								
Football season					1.00	---	1.00	---
Post-season								
MRSA					1.37	0.88, 2.11	1.39	0.90, 2.14
MSSA					0.90	0.64, 1.29	0.91	0.64, 1.30
Pre-season								
MRSA					3.13	1.94, 5.04	3.18	1.97, 5.13
MSSA					1.30	0.87, 1.93	1.31	0.88, 1.96
Spring training								
MRSA					1.34	0,75, 2.42	1.35	0.75, 2.43
MSSA					0.34	0.19, 0.63	0.34	0.19, 0.63

[a]: Adjusted for gender, race (African-American, Caucasian, other), year (Freshman, Junior, Sophomore, Senior, trainer), type of sport (contact vs. non contact)
[b]: Adjusted for race and year

To assess whether colonization with MSSA and MRSA differed across seasons in the football team, we restricted the previous analysis to only those from the football team (1,362 observations from 125 subjects), and we additionally compared the odds of colonization during the athletic seasons. When looking at the football team, the results were similar, except that the confidence intervals for the odds ratio of having MRSA in the summer as compared to winter slightly crossed the null value (Table 13). When looking at the athletic seasons within the football team, during the pre-season, when the team starts training together after the summer break, the odds for colonization with MRSA were significantly higher than during the football season (OR: 3.18; 95% CI: 1.97, 5.13). On the contrary, the odds of colonization with MSSA during spring training were significantly lower than during the football season (OR: 0.34; 95% CI: 0.19, 0.63).

Two sensitivity analyses were performed for the previous models; one adjusted for the number of months subjects were swabbed, categorized as ≤5, >5 and < 13, and ≥13 months; the second excluded subjects swabbed ≤5 months. Results from both analyses remained virtually the same (Table 26 and Table 27, Appendix B).

Prevalence of colonizing sub-types of MRSA and their characteristics

To describe the types of isolates that colonize athletes in this cohort, we evaluated the molecular characteristics of all MRSA isolates recovered during the study. A total of 603 MRSA isolates were recovered from 3,291 observations from 377 subjects. SCC*mec* type IV was present in almost 75% of MRSA isolates (452 of 603), followed by type II with 15% (91 isolates) and type V with 5.8% (35 isolates) (Figure 12). Seven isolates had composite SCC*mec* types.

There were 30 MRSA isolates (4.98%) from six individuals that carried the genes that code for

PVL; of these, 96.7% were USA300. Of 90 USA300 MRSA isolates, 32% were PVL positive and

93% carried SCC*mec* type IV.

All 12 USA PFGE types were represented in this cohort (Figure 12). The most common USA

type was USA200, in almost 20% of MRSA (119 of 603 isolates) isolates, followed by USA300

in almost 15% (90 isolates), USA400 with 12.3% (74 isolates, USA900 with 10.3% (62 isolates)

and USA800 with 10.1% (61 isolates). The least common was USA1200, representing only 2

isolates.

To assess whether the types of MRSA that colonize contact sports athletes differ from those that

colonize noncontact sports athletes, we compared the frequency of USA300 MRSA isolates in

both groups of athletes. The 90 USA300 MRSA isolates were recovered from 30 contact sports

athletes and 4 noncontact sports athletes. Of 2341 contact sports swabs, 76 swabs (3.25%) from

30 subjects had MRSA USA300, while for noncontact sports, 14 of 950 swabs (1.47%) from only

four subjects had MRSA USA300. In other words, 2.61% of all athletes from noncontact sports

had at least one USA300 MRSA isolate during the study, while 13.39% of all athletes in contact

sports carried at least one such isolate (Figure 13; chi-squared $p<0.001$). This means that the

proportion of USA300 MRSA carriage was significantly higher in those who belong to contact

sports teams than for those in noncontact sports. Furthermore, from the 30 contact sports athletes

with USA300 MRSA, 24 belonged to the football team, and 68 USA300 MRSA isolates were

recovered from them (Figure 13); 23 of these subjects had MRSA isolates that carried SCC*mec*

IV in addition of being type USA300. Thus, from the 90 USA300 MRSA isolates, 75.56% were

obtained from the football team alone.

A
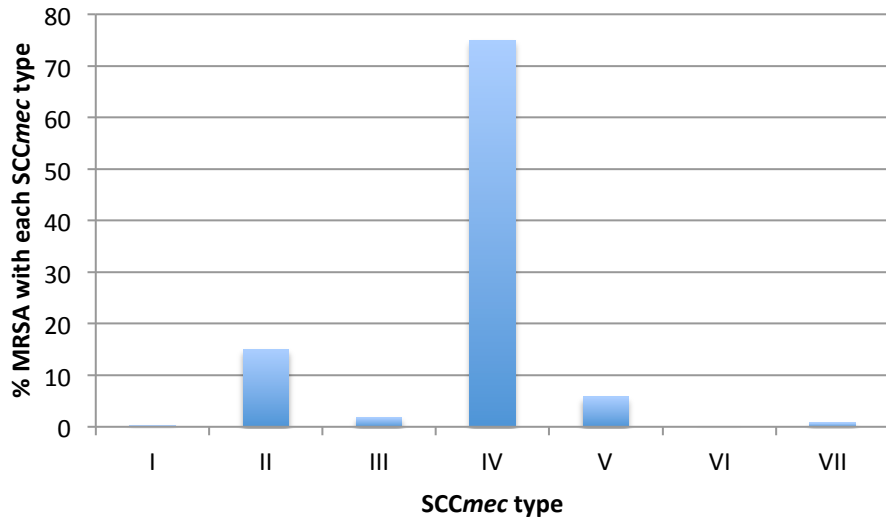
B
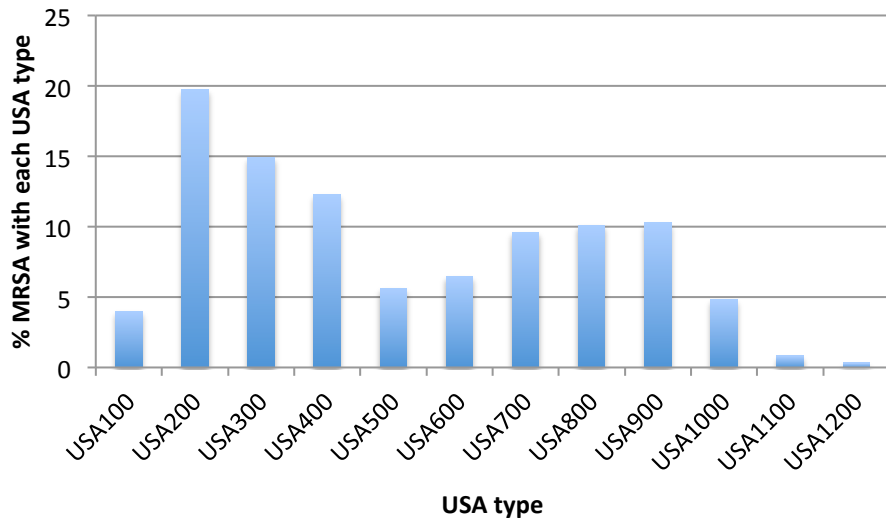
Figure 12. Frequency of SCC*mec* types (A) and USA types (B) among 603 MRSA isolates
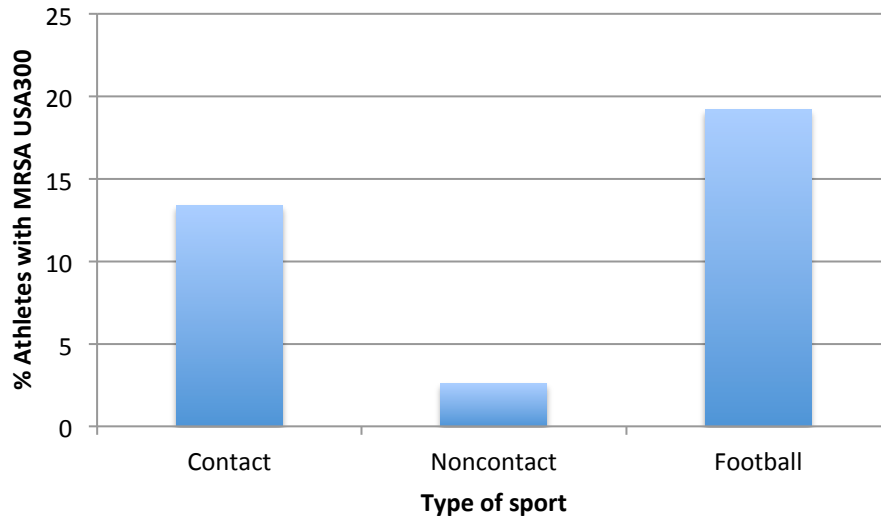
Figure 13. Proportion of athletes with at least one USA300 MRSA isolate by type of sport.

Prevalence of MRSA colonization and incidence of infections in the football team

To identify whether staphylococcal colonization confers a higher risk of infection, we assessed all skin and soft tissue infections that were consistent with staphylococcal disease. Overall, 10 individuals had 12 abscesses. Two infections occurred in February of 2009, while the other 12 occurred between July and September of the same year. All but one of the individuals were on the football team. Of the abscesses, three did not grow *S. aureus*, two grew MSSA (USA1000 and USA700), and seven grew MRSA. All the MRSA isolates carried SCC*mec* type IV and the genes that code for PVL, but one isolate was USA1100, while the other six were USA300.

Though no colonization data were collected from the football team in the three months prior to the larger cluster of cases due to summer vacation, it is noteworthy that the staphylococcal colonization prevalence in the football team in July 2009 was almost 60% (MSSA and MRSA) and colonization with MRSA was 30%; in August, these were 51% and 31%, respectively. In

contrast, for noncontact sports, the frequency of staphylococcal colonization in August was 20%, overall, and 10% for MRSA. All football team members were decolonized at the end of August using mupirocin 2% ointment (twice daily for 5 days) and chlorhexidine 4% solution showers; as a result, in September, the prevalence decreased to 49% for all staphylococcal colonization and 20% for MRSA.

Though there were too few infections to evaluate formally the association between colonization and infection, eight of the 10 infected individuals were colonized with MSSA and MRSA at some point during the study; one was only colonized with MSSA, and the other was never colonized. Five of the nine staphylococcal abscesses occurred while the individuals were colonized; there was no colonization data for two abscesses in one individual.

CHAPTER IV

RESULTS: TIME TO BECOMING COLONIZED WITH *S. AUREUS*

To assess the time it took for athletes who were not colonized at baseline to become colonized with SA, we performed a time-to-event analysis, comparing contact and noncontact sports athletes. Of the 377 enrolled subjects, 191 (50.66%) were already colonized with SA at enrollment, and thus, were truncated and excluded from this analysis (data not shown). Table 14 shows the frequencies of demographics and medical history of subjects included in this analysis. The median time to becoming colonized with SA in the 186 individuals included in the time-to-event analysis was 10 months. For those athletes in contact sports, the median time to SA colonization was 7 months (Figure 14). For those in noncontact sports, the median time to colonization was 17 months (p=0.0293; Figure 14).

Table 14. Univariate analysis for a total prospective cohort of 377 collegiate student athletes, and the left-truncated cohort of 186 athletes after excluding those colonized at baseline

Variable	Total Cohort	Left-truncated Cohort	Left-truncated Freshmen
	N = 377	**N = 186**	**N = 79**
	N (%)	**N (%)**	**N (%)**
Sport			
W. Track and Field	11(2.92)	7 (3.76)	4 (5.06)
W. Tennis	10 (2.65)	6 (3.23)	2 (2.53)
W. Swimming	23 (6.10)	16 (8.60)	10 (12.66)
W. Soccer	29 (7.69)	12 (6.45)	5 (6.33)
W. Lacrosse	34 (9.02)	20 (10.75)	12 (15.19)
W. Cross Country	14 (3.71)	8 (4.30)	4 (5.06)
W. Bowling	15 (3.98)	12 (6.45)	4 (5.06)
W. Basketball	19 (5.04)	9 (4.84)	5 (6.33)
M. Tennis	12 (3.18)	10 (5.38)	4 (5.06)
M. Golf	10 (2.65)	6 (3.23)	1 (1.27)
M. Football	125 (33.16)	48 (25.81)	18 (22.78)
M. Cross Country	10 (2.65)	4 (2.15)	3 (3.80)
M. Basketball	17 (4.51)	6 (3.23)	1 (1.27)
M. Baseball	36 (9.55)	15 (8.06)	6 (7.59)
Trainers	12 (3.18)	7 (3.76)	

Table 14, continued…

Variable	Total Cohort	Left-truncated Cohort	Left-truncated Freshmen
	N = 377	N = 186	N = 79
	N (%)	N (%)	N (%)
Categorized Sports			
Noncontact	153 (40.58)	91 (48.92)	38 (48.10)
Contact	224 (59.42)	95 (51.08)	41 (51.90)
Gender			
Male	216 (57.29)	92 (49.46)	33 (41.77)
Female	161 (42.71)	94 (50.54)	46 (58.23)
Race/Ethnicity			
Caucasian	280 (74.27)	141 (75.81)	57 (72.15)
African-American	80 (21.22)	38 (20.43)	19 (24.05)
Other	17 (4.51)	7 (3.76)	3 (3.80)
College Year			
Freshman	149 (39.52)	79 (42.47)	79 (100.00)
Sophomore	87 (23.08)	41 (22.04)	
Junior	70 (18.57)	34 (18.28)	
Senior	59 (15.65)	25 (13.44)	
N/A	12 (3.18)	7 (3.76)	
Previous surgeries			
No	275 (72.94)	140 (75.27)	59 (74.68)
Yes	102 (27.06)	46 (24.73)	20 (25.32)
History of SA infection			
No	355 (94.16)	179 (96.24)	78 (98.73)
Yes	22 (5.84)	7 (3.76)	1 (1.27)
SA infection in contacts			
No	261 (69.23)	127 (68.28)	53 (67.09)
Yes	116 (30.77)	59 (31.72)	26 (32.91)
Comorbidities			
No	362 (96.02)	180 (96.77)	75 (94.94)
Yes	15 (3.98)	6 (3.23)	4 (5.06)
Antibiotics in Previous 6 Months			
No	329 (87.27)	156 (83.87)	66 (83.54)
Yes	48 (12.73)	30 (16.13)	13 (16.46)
SA Colonization			
Never SA	90 (23.87)	90 (48.39)	39 (49.37)
Ever SA	287 (76.13)	96 (51.61)	40 (50.63)

W: women's team

M: men's team
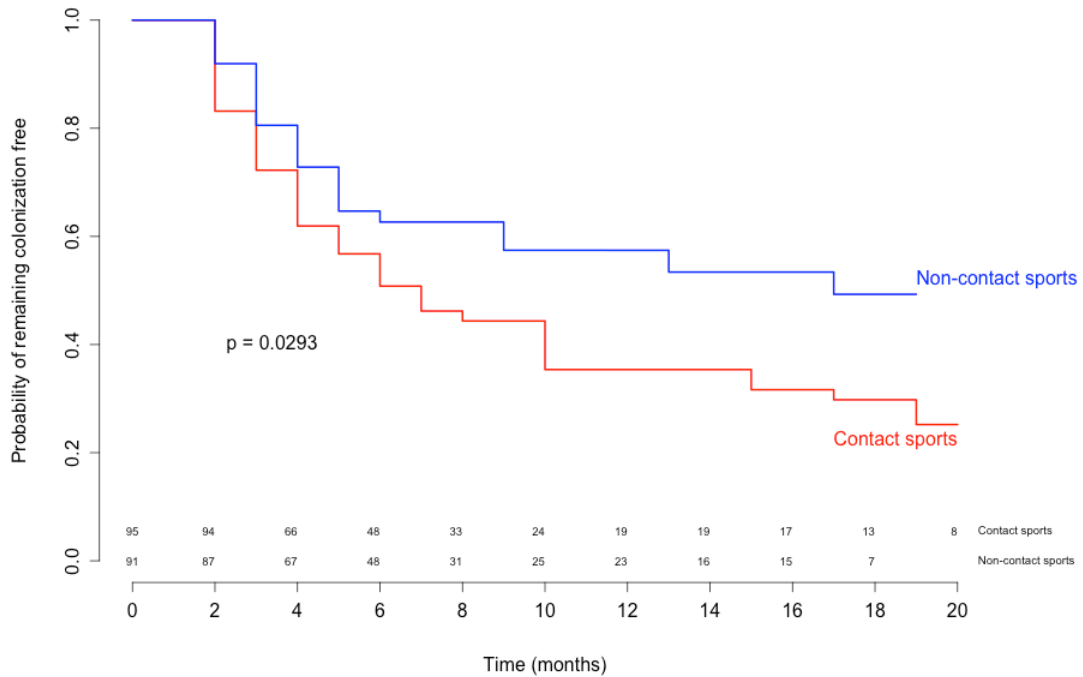
Figure 14. Kaplan-Meier curve for the time to becoming colonized with *S. aureus* in a cohort of 186 college athletes, by whether or not they participate in contact sports

Based on a multivariate parametric survival model, adjusting for gender, race, history of staphylococcal infections, and season of enrollment, and assuming a Weibull distribution, the hazard of becoming colonized with SA for athletes who participate in contact sports was 1.6 times higher than for those athletes who participate in noncontact sports (95% CI: 1.02 and 2.5; Table 15). Other variables included in the model were not statistically significant.

Table 15. Hazard ratios for crude and adjusted models

	All athletes		Freshmen only	
	Crude Model	Adjusted Model[a]	Crude Model	Adjusted Model[b]
	HR (95% CI)	HR (95% CI)	HR (95% CI)	HR (95% CI)
Noncontact sports	1.00 (---)	1.00 (---)	1.00 (---)	1.00 (---)
Contact sports	1.55 (1.05, 2.31)	1.61 (1.02, 2.55)	1.92 (1.06, 3.50)	1.89 (1.04, 3.44)

[a] Adjusted for gender, race, history of staphylococcal infections, and season of enrollment
[b] Adjusted for gender, race

When looking at how much each variable included in the model contributed in explaining the observed differences in the time to becoming colonized with *S. aureus*, most of these differences were explained by whether athletes participated in either contact or noncontact sports (Figure 15). The residual plots indicated that the model and its assumed Weibull distribution adequately fitted all variables included in the multivariate model (data not shown).

A   B
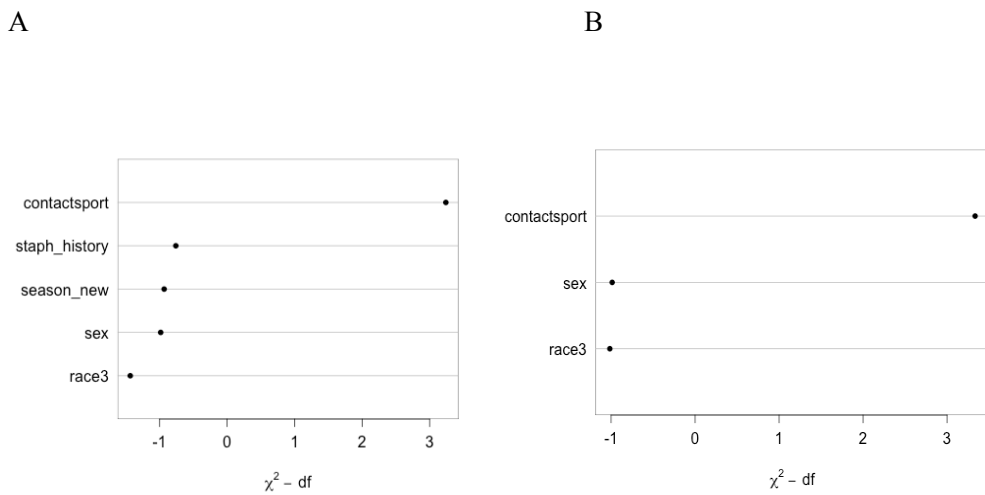
Figure 15. Contribution of each covariate in explaining the hazards of becoming colonized with SA. A: model includes athletes from all college years. B: model includes only freshmen.

To assess whether freshmen, who are just joining their athletic teams, presented a similar pattern in their time to becoming colonized, a similar time-to-event analysis as the one described above was performed, but restricting it to those freshmen free of colonization at baseline. The median time to colonization in freshmen was 10 months. For those freshmen in contact sports, the median time to colonization was 6 months (Figure 16); for those in noncontact sports the median time to colonization was >20 months (p=0.0317; Figure 16). The hazard of becoming colonized with SA for freshmen who participate in contact sports was 1.9 times higher than for those freshmen who participate in noncontact sports (95% CI: 1.04 and 3.44; Table 15).
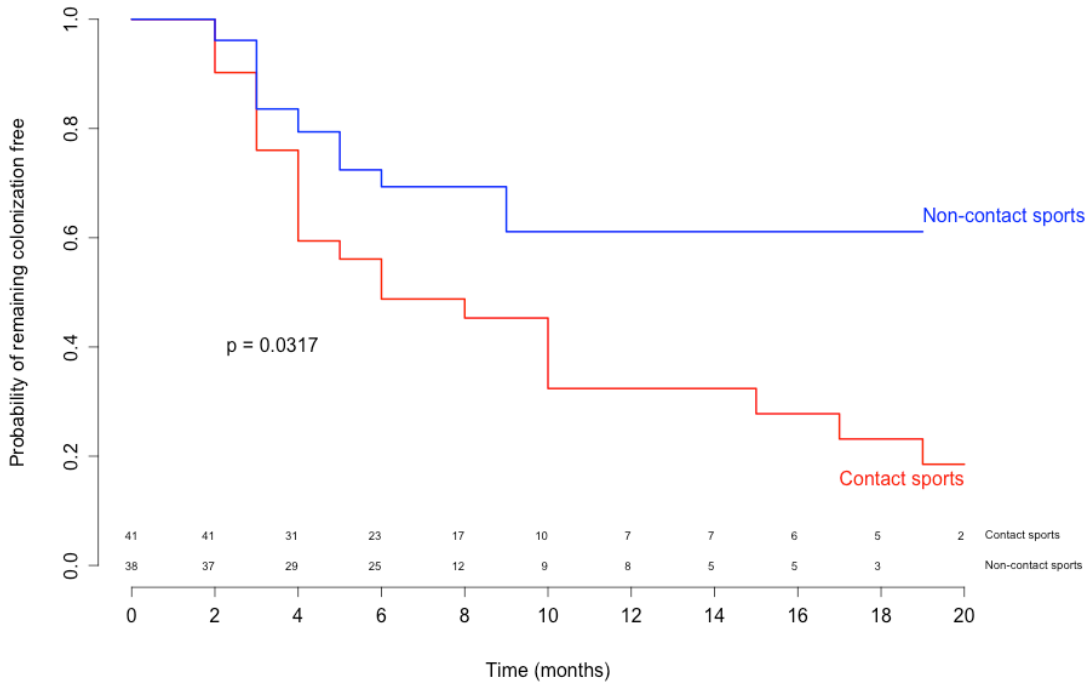
Figure 16. Kaplan-Meier curve for the time to becoming colonized with *S. aureus* in a cohort of 79 freshmen athletes, by whether or not they participate in contact sports

Two sensitivity analyses were performed for the previous models; one adjusted for the number of months subjects were swabbed, categorized as ≤5, >5 and < 13, and ≥13 months; the second excluded subjects swabbed ≤5 months. Results from both analyses became not significant (Table 28 and Figure 17, Appendix B). These analyses, however, decreased the power to detect a significant difference in the time to becoming colonized with SA by increasing the degrees of freedom and by decreasing the sample size, respectively. Additionally, excluding subjects with shorter follow-up time in a time-to-event model would also remove individuals who became colonized in a very short time, limiting the statistical power to detect a small difference in colonization times.

CHAPTER V

RESULTS: RISK FACTORS FOR LONGITUDINAL COLONIZATION

Specific Aim #2

To model the association between colonization with CA-MRSA in athletes and the type of sport they participate in, demographic, medical history variables and CA-MRSA subtype, as compared to colonization with methicillin-susceptible *S. aureus* (MSSA) or no colonization, while adjusting for potential confounders and assessing effect measure modifiers.

Colonization and Sport

To assess risk factors associated with staphylococcal colonization, demographic and medical history data from each subject's baseline questionnaire was linked to their colonization status over time. Table 16 shows the bivariate analysis for demographic and medical history by type of sport. A multinomial mixed model with random intercepts was built. Based on the unadjusted model, contact sports athletes had higher odds of being colonized with MRSA and MSSA than athletes in noncontact sports teams (Table 17). Based on the adjusted model, those athletes in contact sports had significantly higher odds of being colonized with MRSA than not being colonized when compared to those athletes in noncontact sports (OR: 2.36; 95% CI: 1.13, 4.93; Table 17). Though higher odds of MSSA colonization in contact sports athletes was also suggested, the result was borderline significant (OR: 1.57; 95% CI: 0.94, 2.62). Having had previous staphylococcal infections was also associated with increased odds of MRSA carriage in this cohort (OR: 5.35; 95% CI: 1.51, 18.87). Sophomores were more likely to carry MSSA than

not being colonized at all when compared to freshmen (OR: 1.79; 95% CI: 1.02, 3.14). Females

(OR: 0.26; 95% CI: 0.13, 0.50), African-Americans (OR: 0.35; 95% CI: 0.15, 0.79), and those

whose contacts had a previous staphylococcal infection (OR: 0.45; 95% CI: 0.22, 0.94) had less

odds of being colonized with MRSA than not being colonized with SA when compared to males

and Caucasians, respectively. No other significant associations between covariates and

colonization were found. The multiplicative interaction between contact sport and gender was not

statistically significant (data not shown).

Three sensitivity analyses were performed for the previous models; one adjusted for the number

of months subjects were swabbed, categorized as ≤5, >5 and < 13, and ≥13 months; the second

excluded subjects swabbed ≤5 months; and the third model categorized baseball players as

contact sports athletes instead of noncontact. Results from all three analyses did not virtually

change (Table 29 and Table 30, Appendix B).

Table 16. .Bivariate analysis for sport, demographics and medical history for a cohort of 377 college student athletes at Vanderbilt University, by type of sport

Variable	Total Cohort N=377 N (%)	Noncontact N=153 N (%)	Contact N=224 N (%)
Gender			
Female	161 (42.71)	79 (51.63)	82 (36.61)
Male	216 (57.29)	74 (48.37)	142 (63.39)
Race/Ethnicity			
Caucasian	280 (74.27)	133 (86.93)	147 (65.62)
African-American	80 (21.22)	11 (7.19)	69 (30.80)
Other	17 (4.51)	9 (5.88)	8 (3.57)
College Year			
Freshman	149 (39.52)	64 (41.83)	85 (37.95)
Sophomore	87 (23.08)	36 (23.53)	51 (22.77)
Junior	70 (18.57)	23 (15.03)	47 (20.98)
Senior	59 (15.65)	18 (11.76)	41 (18.30)
Trainers	12 (3.18)	12 (7.84)	0 (0.00)
History of SA infection			
No	355 (94.16)	148 (96.73)	207 (92.41)
Yes	22 (5.84)	5 (3.27)	17 (7.59)
SA infection in contacts			
No	261 (69.23)	122 (79.74)	139 (62.05)
Yes	116 (30.77)	31 (20.26)	85 (37.95)
Previous surgeries			
No	275 (72.94)	126 (82.35)	149 (66.52)
Yes	102 (27.06)	27 (17.65)	75 (33.48)
Comorbidities			
No	362 (96.02)	148 (96.73)	214 (95.54)
Yes	15 (3.98)	5 (3.27)	10 (4.46)
Antibiotics in Previous 6 Months			
No	329 (87.27)	132 (86.27)	197 (87.95)
Yes	48 (12.73)	21 (13.73)	27 (12.05)

Table 17. Odds ratios for multinomial mixed models with random intercept

	Unadjusted		Adjusted	
	OR	95% CI	OR	95% CI
Noncontact sports	1.00	---	1.00	---
Contact sports				
MRSA	2.25	1.14, 4.43	2.36	1.13, 4.93
MSSA	1.68	1.06, 2.65	1.57	0.94, 2.62
Male			1.00	---
Female				
MRSA			0.26	0.13, 0.50
MSSA			0.80	0.50, 1.28
Caucasian			1.00	---
African-American				
MRSA			0.35	0.15, 0.79
MSSA			0.77	0.44, 1.34
Other race/ethnicity				
MRSA			0.46	0.09, 2.31
MSSA			1.39	0.47, 4.15
Freshman			1.00	---
Sophomore				
MRSA			1.25	0.53, 2.79
MSSA			1.79	1.02, 3.14
Junior				
MRSA			1.25	0.54, 2.90
MSSA			1.69	0.94, 3.04
Senior				
MRSA			1.07	0.40, 2.81
MSSA			1.20	0.61, 2.37
Trainers				
MRSA			0.97	0.14, 6.69
MSSA			0.82	0.21, 3.27
No previous SA infections			1.00	---
Previous staphylococcal infections				
MRSA			5.35	1.51, 18.87
MSSA			2.19	0.86, 5.52
No SA infections in contacts			1.00	---
Staphylococcal infections in contacts				
MRSA			0.45	0.22, 0.94
MSSA			0.72	0.43, 1.20

Table 17, continued

	Unadjusted		Adjusted	
	OR	95% CI	OR	95% CI
No comorbidities			1.00	---
Any comorbidities				
MRSA			2.90	0.65, 13.01
MSSA			1.71	0.59, 5.01
No previous surgeries			1.00	---
Any Previous surgeries				
MRSA			1.28	0.63, 2.60
MSSA			1.31	0.80, 2.15
No previous antibiotics			1.00	---
Previous antibiotics				
MRSA			1.29	0.50, 3.34
MSSA			0.59	0.30, 1.18

[a] Adjusted for gender, race (African-American, Caucasian, other), year (Freshman, Junior, Sophomore, Senior, trainer), history of staphylococcal infections, staphylococcal infections in contacts, any comorbidities, previous surgeries, previous antibiotics, contact sport – gender interaction, and time

CHAPTER VI

RESULTS: ASSOCIATION BETWEEN CARRIER PROFILES AND SPORT, TYPE OF ISOLATE AND COLONIZATION SITE

Specific Aim #3

To model the association between persistent staphylococcal colonization and type of sport, site of colonization and specific staphylococcal virulence factors, as compared to intermittent colonization or no colonization with *S. aureus*, while adjusting for potential confounders.

Estimating the Association Between Persistent Staphylococcal Carriage and Sport

To assess whether the frequency of persistent, intermittent and never carriers of SA differed by the type of sport athletes participate in, we used each subject's colonization results over time to categorize subjects into either of the three carrier profiles, and we linked these results to their demographic characteristics and medical history, using a multinomial logistic regression model. As indicated in Table 18, approximately 61% of the individuals who never carried SA belonged to noncontact sports teams, while 68% of those who intermittently carried SA and 60% of those who persistently carried SA belonged to contact sports teams. Males also represented the majority of intermittent and persistent carriers, while women were the majority among non-carriers.

Table 18. Bivariate analysis for sport, demographics and medical history for a cohort of 377 college student athletes at Vanderbilt University, by overall colonization status

Variable	Total Cohort N=377 N (%)	Never carriers N (%) 90 (23.87)	Intermittent carriers N (%) 200 (53.05)	Persistent carriers N (%) 87 (23.08)
Categorized Sports				
Noncontact	153 (40.58)	55 (61.11)	63 (31.50)	35 (40.23)
Contact	224 (59.42)	35 (38.89)	137 (68.50)	52 (59.77)
Gender				
Female	161 (42.71)	48 (53.33)	85 (42.50)	28 (32.18)
Male	216 (57.29)	42 (46.67)	115 (57.50)	59 (67.82)
Race/Ethnicity				
Caucasian	280 (74.27)	68 (75.56)	145 (72.50)	67 (77.01)
African-American	80 (21.22)	18 (20.00)	45 (22.50)	17 (19.54)
Other	17 (4.51)	4 (4.44)	10 (5.00)	3 (3.45)
College Year				
Freshman	149 (39.52)	39 (43.33)	81 (40.50)	29 (33.33)
Sophomore	87 (23.08)	16 (17.78)	48 (24.00)	23 (26.44)
Junior	70 (18.57)	14 (15.56)	42 (21.00)	14 (16.09)
Senior	59 (15.65)	16 (17.78)	25 (12.50)	18 (20.69)
Trainers	12 (3.18)	5 (5.56)	4 (2.00)	3 (3.45)
History of SA infection				
No	355 (94.16)	87 (96.67)	186 (93.00)	82 (94.25)
Yes	22 (5.84)	3 (3.33)	14 (7.00)	5 (5.75)
SA infection in contacts				
No	261 (69.23)	61 (67.78)	132 (66.00)	68 (78.16)
Yes	116 (30.77)	29 (32.22)	68 (34.00)	19 (21.84)
Previous surgeries				
No	275 (72.94)	74 (82.22)	137 (68.50)	64 (73.56)
Yes	102 (27.06)	16 (17.78)	63 (31.50)	23 (26.44)
Comorbidities				
No	362 (96.02)	88 (97.78)	190 (95.00)	84 (96.55)
Yes	15 (3.98)	2 (2.22)	10 (5.00)	3 (3.45)
Antibiotics in Previous 6 Months				
No	329 (87.27)	78 (86.67)	172 (86.00)	79 (90.80)
Yes	48 (12.73)	12 (13.33)	28 (14.00)	8 (9.20)

Based on an unadjusted multinomial logistic model, those athletes in contact sports had higher

odds of being intermittently or persistently colonized than never being colonized, when compared

to noncontact sports athletes (Table 19; OR: 3.42; 95%CI: 2.03, 5.74; and OR: 2.33; 95% CI:

1.28, 4.27, respectively). The observed association between intermittent and persistent carriers

and type of sport remained statistically significant after adjusting for gender, race and college

year (Table 19; OR: 3.60; 95%CI: 2.02, 6.40; and OR: 2.39; 95% CI: 1.21, 4.72, respectively).

These results indicate that athletes in contact sports teams not only tend to have higher prevalence

of staphylococcal colonization on average, but also tend to be colonized for longer periods of

time than athletes in noncontact sports teams. Results from two sensitivity analyses, adjusting for

the number of months subjects were swabbed and excluding subjects swabbed ≤5 months, were

not materially different (Table 31, Appendix B).

Table 19. Unadjusted and adjusted multinomial logistic regression models comparing the odds of being persistent, intermittent or never SA carriers between 377 contact and noncontact sports athletes

	Crude Model		Adjusted Model[a]	
	OR	95% CI	OR	95% CI
Contact sports				
Never SA carriers	1.00	---	1.00	---
Intermittent SA carriers	3.42	2.03, 5.74	3.60	2.02, 6.40
Persistent SA carriers	2.33	1.28, 4.27	2.39	1.21, 4.72

[a]: Adjusted for gender, race, and college year

Estimating the Association Between Persistent Staphylococcal Carriage and Type of

Colonizing MRSA Isolate

To examine if MRSA isolates with particular characteristics tend to be more likely to cause either

persistent or intermittent colonization, we linked the carrier profile with the type of MRSA

colonizing isolate, and performed a logistic regression model. Athletes who were never colonized

were excluded from these analyses. Table 20 shows that the number of athletes who had at least

one MRSA isolate with SCC*mec* type IV was significantly higher among persistent

staphylococcal carriers than among intermittent carriers. Also, all athletes who carried PVL-

encoding MRSA isolates were intermittent carriers; thus, PVL predicted the carrier profile

perfectly, and it was not considered further in the logistic regression models. When performing

multivariate logistic regression models, adjusting for gender, race and type of sport, no

association was observed between athletes who had at least one MRSA USA300 isolate and their

carrier profile (Table 21), but those athletes who carried at least one MRSA isolate with SCC*mec*

type IV had significantly higher odds of being persistent carriers than intermittent carriers (Table

21; OR: 5.30; 95% CI: 2.88, 9.77). Sensitivity analyses adjusting for the number of months

subjects were swabbed and excluding subjects swabbed ≤5 months did not materially change the

results (Table 32, Appendix B).

Table 20. Individuals who ever had isolates with the following characteristics

	Total cohort N (%) N = 377	Intermittent carriers N (%) N = 200	Persistent carriers N (%) N = 87	P
SCC*mec* IV	152 (40.32)	85 (42.50)	67 (77.01)	<0.001[a]
USA300	32 (8.49)	21 (10.50)	11 (12.64)	0.596[a]
PVL positive	6 (1.59)	6 (3.00)	0 (0.00)	0.183[b]

[a]: Pearson's chi-squared
[b]: Fisher's exact test

Table 21. Logistic regression models comparing the odds of being persistent or intermittent SA
carriers by isolate type between 287 athletes (excluding those who were never SA carriers)

	Crude Model		Adjusted Model[a]		Adjusted Model[b]	
	OR	95% CI	OR	95% CI	OR	95% CI
USA300 vs. other						
Intermittent SA carriers	1.00	---	1.00	---	1.00	---
Persistent SA carriers	1.23	0.57, 2.69	1.19	0.53, 2.66	0.68	0.30, 1.56
SCC*mec* IV vs. other						
Intermittent SA carriers	1.00	---	1.00	---	1.00	---
Persistent SA carriers	4.53	2.55, 8.04	5.01	2.75, 9.11	5.30	2.88, 9.77

[a]: Adjusted for sport (contact vs. noncontact), gender and race
[b]: Adjusted for sport (contact vs. noncontact), gender, race, USA300, and SCC*mec* IV

Estimating the Association Between Persistent Staphylococcal

Carriage and Colonization Site

To examine whether subjects who carry SA intermittently or persistently tend to be colonized in different body sites, the site of colonization over time was linked to the carriage profile, using a multinomial mixed model with random intercepts. Athletes who were never colonized were excluded from this analysis. Table 22 shows that the odds of oropharyngeal carriage alone are significantly lower than for nasal carriage alone for persistent carriers, as compared to intermittent carriers, even after adjusting for potential confounders (OR: 0.16; 95% CI: 0.08, 0.31). On the other hand, persistent carriers are more likely than intermittent carriers to have both nasal and oropharyngeal colonization than to have nasal colonization alone (OR: 2.05; 95% CI: 1.39, 3.02). These results suggest that carrier profiles might not only differ in their duration of colonization with SA, but might also differ in the body sites that harbor SA. Results from sensitivity analyses, adjusting for the number of months subjects were swabbed and excluding subjects swabbed ≤5 months, were not materially different (Table 33, Appendix B).

Table 22. Odds ratios for colonization site by carrier profile in 287 subjects, based on multinomial mixed models with random intercept (excluding those who were never colonized)

Colonization Site/Type	Crude Model		Adjusted Model[a]	
	OR	95% CI	OR	95% CI
Intermittent carriers	1.00	---	1.00	---
Persistent carriers				
Nasal only	1.00	---	1.00	---
Nasal and oropharyngeal	2.05	1.40, 3.02	2.05	1.40, 3.02
Oropharyngeal only	0.16	0.08, 0.31	0.16	0.08, 0.31

[a]: Adjusted for contact sport (contact vs. noncontact), gender, race, college year

CHAPTER VII

DISCUSSION AND SUMMARY

In this, the largest prospective cohort study to date of healthy collegiate athletes, we demonstrated that colonization with SA is dynamic over time, and that athletes who are part of contact sports teams become colonized more rapidly, are at higher odds of being colonized over time, and have higher odds of being intermittent and persistent staphylococcal carriers when compared to athletes in noncontact sports.

Most previous reports have focused on contact sports and male athletes.[93,117] To assess the epidemiology of staphylococcal colonization over time, we performed a comprehensive longitudinal assessment of colonization in a collegiate athlete population, including 14 different sports and 43% females. Most previous reports in athletic populations had also found less than 20% prevalence of MRSA colonization, with many finding no MRSA carriers. [11,25,94,96,98,101-103,107,108,116,118,119] In this diverse cohort, however, a high proportion of athletes were colonized at some point during the study: 76% with any SA, and 46% with MRSA. Staphylococcal colonization varied over time, ranging, at any single month, from 34% to 62% overall, from 18% to 42% for MSSA, and from 8% to 29% for MRSA (Figure 7). Though it has been recognized that athletes tend to have higher prevalence of colonization with MRSA (5.4%) than the general population (1.3%), [10] the MRSA prevalence in the current study is even higher than a previous report from our group, where the MRSA prevalence in the football team ranged from 4% to 16%,[94] while in the current study it ranged from 7% to 31% (Figure 9). Previous studies in pediatric populations from this same region have also reported an increase in the prevalence of MRSA carriage, from 0.8% in 2001[41] to 9.2% in 2004.[16] This increase in MRSA prevalence is

alarming, since MRSA is a leading cause of both SSTIs and invasive infections,[15] and MRSA

infections have worse outcomes,[4,19-21] such as longer lengths and higher costs of hospitalization

than MSSA infections.[22]

Nasal and oropharyngeal colonization were assessed to increase our sensitivity in detecting

colonized individuals and to compare staphylococcal colonization in different body sites. The

combination of nasal and oropharyngeal swabs is said to detect 85.4% of colonized individuals.[45]

Like overall colonization, nasal and oropharyngeal colonization prevalence also varied over time.

SA colonization in both body sites, however, did not seem to follow a parallel pattern: nasal

colonization did not increase when oropharyngeal colonization increased, and vice versa. These

differing colonization patterns suggest that SA can colonize one body site without necessarily

affecting colonization in other body sites. Nonetheless, similar to what has been reported, the

prevalence of nasal carriage was higher than for oropharyngeal carriage.[5,44] The nose is

commonly the body site with the highest colonization rate and the one that yields the highest

bacterial load.[5,44,45] Though during one month the oropharyngeal SA carriage prevalence was

47%, higher than the nasal prevalence, nasal SA colonization prevalence was higher on average

(Figure 11). Interestingly, MSSA seemed to be more commonly colonizing the oropharynx than

the nares (Figure 10), while the converse was true for MRSA.

It was interesting to find that SA seemed to preferentially colonize individuals in one body site

over another. Athletes with exclusive nasal colonization one month were more likely to have

nasal colonization again the next month when compared to athletes with no colonization the

previous month (Table 11). Athletes colonized in both sites were also more likely to be colonized

in both sites again. These results suggest that SA might preferentially colonize the nose as

opposed to the oropharynx, which goes back to the nose being the most commonly colonized body site.[5,44]

Though axillary colonization was also assessed during the first five months of the study, it did not increase our sensitivity in detecting colonized athletes: less than 2% of the individuals swabbed in the first five months were colonized exclusively in the axilla. Given the low detection, it was decided that the costs outweighed the small number of subjects with exclusive axillary colonization, and axillary colonization was not further analyzed nor included in any of the analyses presented. Begier and colleagues identified only one football player with axillary but not nasal colonization,[96] and Stacey and colleagues did not find any axillary colonization, though the number of athletes was small.[103] This low axillary colonization in previous reports supports our decision to discontinue obtaining axillary swabs after the first five months of the study. The lack of axillary swabs after the fifth month represents a potential for misclassification of colonized athletes. This misclassification, however, would not likely have a significant impact on our results, since 76% of the cohort had contributed axillary swabs, and only 1.7% would have been misclassified. Further, of the five subjects who had exclusive axillary colonization, three of them belonged to contact sports teams and one of them had axillary colonization during two samplings. Thus, had they been considered as colonized in the analyses, the association between the type of sport and colonization would have been stronger than what was actually calculated. Additionally, besides being a less frequently colonized site when compared to the nares and oropharynx,[5] axillary colonization tends to also yield the lowest bacterial load among several sampled body sites.[45]

Though it is considered that there is no seasonality in SA colonization,[44] we observed significantly different odds of colonization by season, both for the full cohort and for the football

team alone. All athletes were more likely to be colonized with SA in the summer than in the winter, and less likely to be colonized with MRSA in the fall than in the winter. This seasonality in collegiate athletes might be explained by the school and training cycle, with colonization increasing when the teams start practicing after the summer vacation. As for the odds in seasonal colonization for the full cohort being highest in the summer and lowest in the fall when compared to winter colonization, these results are most likely influenced by the colonization in the football team, since this was the largest team in the cohort. In terms of football seasons, the highest odds of having MRSA occurred in the pre-season (July-August) when compared to the football season (September-November), while the lowest odds of having MSSA were during spring training (March-June). Our previous study had shown that colonization prevalence with SA and MRSA in the football team was highest during the football season (August-October), and was lowest during the off-season (May-July).[94] This slight discrepancy in results for the football team can be explained by two facts. First, the football seasons change slightly from year to year, depending on the timing of school vacation and class cycles. Thus, the athletic seasons for the current study were categorized differently than for the previous study. The pre-season in the current study included July and August; on the previous study, August was included in the football season. In the current study, August of 2008 and 2009 were some of the months with the highest colonization prevalence (Figure 7 and Figure 9) and thus, this would explain the higher prevalence in the pre-season as opposed to the football season. Second, there was an outbreak in the football team between July and September of the second year in the current study, during which the colonization prevalence in the football team was highest. At the end of August of the same year, all football team athletes received decolonization treatment with mupirocin and chlorhexidine baths, which brought colonization prevalence down for the fall/football season. Additionally, the alarm of infections and hospitalizations in team members might have

heightened the awareness of maintaining good hygiene practices among the team and staff members.

The small outbreak that affected mostly the football team occurred between July and September of 2009. Other reported outbreaks have also occurred in similar seasons, after the start of training camps and beginning of the football season. In an European report, an outbreak of MRSA occurred between July and October,[120] while the University of Florida football team experienced an outbreak between September and December,[121] and another collegiate football team experienced an outbreak between August and September.[27] Our findings of higher colonization prevalence during July and August and a disease outbreak between July and September, along with previous findings suggest that there is actually a potential seasonality in the spread of colonizing and infecting MRSA. Transmission of this organism could be facilitated by the warmer climate during the aforementioned months, which provides a humid and warm environment that allow these bacteria to grow more in the skin and on sports equipment and clothing. In addition, return to training camps, or school activities for high school and collegiate teams, allows for the easier person-to-person transmission from colonized to not colonized individuals. Also, collegiate athletes can also be roommates in school dormitories, which would also facilitate transmission of colonizing and infecting isolates, given that household members where there is a colonized or infected individual have increased risk of also having colonization and infection with MRSA.[24,122] In our study, however, no information was collected on participants' housing arrangements. Future studies in collegiate athletes could explore the influence of athletes housing together in their risk of colonization and infection with MRSA.

In addition to assessing the longitudinal epidemiology of SA colonization, the molecular characteristics of colonizing MRSA isolates were also examined. Of the MRSA isolates obtained,

75% carried SCC*mec* type IV, which is consistent with CA-MRSA strains. A previous study from our group had found an increase in colonizing SCC*mec* IV MRSA isolates in a pediatric population, from 47% in 2003-2006 to 91% in 2006-2008.[81] Our previous, smaller athlete study had found 81% of colonizing isolates were SCC*mec* IV.[94] Whether a similar increase in SCC*mec* type IV in the athletic population has happened would need to be assessed through continuous surveillance cultures.

Though CA-MRSA seemed to be common among colonized athletes, less than 5% of the MRSA isolates carried the genes that code for PVL, a toxin that has also been associated with CA-MRSA. Though the PVL-encoding genes are less commonly found in colonizing strains than in clinical strains, the prevalence of these genes in the current study is far lower than in previous colonization studies; a study from our group had reported 24% of colonizing isolates in a pediatric population were PVL positive,[81] while other study in soldiers reported 58% of the colonizing isolates carried these genes,[4] and even as high as 62% of colonizing isolates have been reported to carry PVL-encoding genes.[46] Nonetheless, our previous study of a smaller cohort of athletes did not find PVL genes in any colonizing isolates. Whether there is a factor that "protects" athletes from being colonized with PVL positive strains would require further study.

The types of MRSA colonizing isolates represented a heterogeneous group of strains (Figure 12), which is consistent with previous findings.[81] Similar to our previous athlete study, the most common MRSA type was USA200, followed by USA300. [94] The prevalence of USA300 colonizing isolates, however, was higher in the current study, changing from 9.4% to 15%.[94] This higher prevalence of USA300 more closely resembles that of a previously studied pediatric population.[81]

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Interestingly, USA300 MRSA more frequently colonized contact sports athletes than noncontact sports athletes: 16.7% of MRSA isolates recovered from contact sports athletes were USA300, while only 9.5% were recovered from noncontact sports athletes. Even more notably, 76% of USA300 MRSA isolates were obtained from the football team alone. The higher prevalence of a more virulent strain among contact sports athletes provides a potential explanation as to why athletes in contact sports, and especially football teams, are often victims of MRSA outbreaks, given that infections can often arise from endogenous colonizing strains.[1,42] These data also suggest that contact sports athletes would be the group where most prevention efforts and strategies should be placed, since it is a population at higher risk of staphylococcal skin and soft tissue infections.

Though colonization prevalence was as high as 62% during the study, there were only nine staphylococcal infections, seven of them due to MRSA, so that a statistical association could not be formally examined due to lack of power. The proportion of athletes colonized during the time of these infections, however was high, reaching 60% for SA and 31% for MRSA alone, and most of the athletes who got a skin and soft tissue infection were also colonized at the time of infection. Additionally, only one infected athlete was not from the football team. Furthermore, six of the infecting SA isolates (66.67%) had characteristics consistent with the epidemic clone: USA300, SCC*mec* type IV, and carried the genes that encode for PVL. With few exceptions,[27,120] most reported outbreaks among athletes have been due to this same strain.[11,25,96,102] Although colonization with SA is usually considered a risk factor for SA infections,[1] reports of outbreaks that have scarcely assessed colonization have found no or very low prevalence of colonization, and have suggested that nasal colonization is not a risk factor for infection in athlete populations,[11,96,118] and is overshadowed by direct person-to-person contact and skin trauma. Most of these studies have assessed colonization after the start of the outbreak, though, when

antimicrobial therapy in addition to hygiene measures and heightened awareness in team members and coaching staff might have underestimated nasal colonization prior to the outbreak.[11,96] Conversely, in our study we assessed colonization prospectively. A high prevalence of both MSSA and MRSA colonization was observed, and 85.71% of the individuals who got a staphylococcal infection had also been colonized with SA in previous months. Due to the small number of infections, however, we could not assess the association between several potential risk factors and infection. These data suggest that there indeed could be a link between these contact sports athletes being colonized and subsequently acquiring an infection with their colonizing strain. A larger study, organized by the NCAA or NFL and including several athlete populations through a consortium of institutions would likely be able to properly assess risk factors for infection in athletes in a prospective manner.

Based on the longitudinal analysis, contact sports athletes had over twice the odds of colonization with MRSA and seemed to also have higher odds of colonization with MSSA when compared to noncontact sports athletes, though the latter was borderline significant after adjusting for confounders. These results suggest that the use of shared equipment, facilities and objects that participating in a sports team often involves does not necessarily increase the risk of colonization, but it is probably the closer and more aggressive contact between teammates that might play a more central role in facilitating colonization of athletic team members, as previously suggested.[117] In athletes, sharing towels and personal items, having skin abrasions or "turf burns", and skin-to-skin contact have been inconsistently found to be risk factors for MRSA infections.[11,96,97] Also, according to the CDC, transmission of CA-MRSA is facilitated by the "5 Cs", all of which are common in athletes: crowding, contact (skin-to-skin), compromised skin, contaminated items, lack of cleanliness. Although we did not assess the 5C's and their association with colonization, it

is likely that these factors contribute not only to spread infections, but also to transmission of colonizing isolates.

Other risk factors for being colonized over time were being male, Caucasian or a sophomore, having had a staphylococcal infection or not having a contact that had a staphylococcal infection previously. Though most of these risk factors have not been assessed in athletic populations, being male, Caucasian and having previous staphylococcal infections are usually considered risk factors for SA.[2,110] Given that household contacts of a colonized or previously infected individual are more likely to have colonization and potential infection,[24,122] we did not expect to find that having contacts with previous staphylococcal infections decreased the odds of colonization in athletes. Whether recall bias was the cause of this unexpected fining could be evaluated through a validation study that would include reviewing medical records of athletes' close contacts. Similarly, whether a racial difference in the prevalence of staphylococcal colonization is due to biological factors of the host or due to behavioral or socioeconomic differences would require a more focused study.

Moreover, those in contact sports became colonized more rapidly than those in noncontact sports. Even when looking only at freshmen who were just joining their respective athletic teams, the hazard ratio for becoming colonized with SA remained similar for contact sports athletes when compared to noncontact sports athletes. Given the long median time to becoming colonized, 17 months for noncontact and 7 months for contact sports, it is not likely that these data could be explained by the higher odds of persistent and intermittent colonization among contact sports athletes. Additionally, even after adjusting for potential confounders, the type of sport was the characteristic that explained most of the difference in the hazard of becoming colonized (Figure 15). Thus, the higher hazard of becoming colonized for athletes in contact sports reinforces our

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finding that athletes who participate in contact sports are at higher risk of staphylococcal colonization.

To further strengthen our results, we also observed a higher percentage of intermittent and persistent staphylococcal carriers among contact sports participants, and a higher percentage of non-carriers among noncontact sports participants. Though we did not obtain weekly swabs, as previous studies that have characterized the three staphylococcal carrier profiles,[3,49] we did have monthly data for 18 months to assess the regularity of carriage in our population. A study by van Belkum and colleagues, however, also assessed these carrier profiles by obtaining nasal samples every three to four weeks, which more closely resembles our sampling periods.[7] Though there is a potential for misclassification into the three carrier profiles, it would likely be non-differential, since it would not have differed systematically by type of sport, by colonization site, or by type of isolate, given the prospective nature of the study. Additionally, the probability of misclassifying those who were intermittent carriers as non-carriers, or persistent carriers as intermittent, and vice versa, would probably be similar. Further, the percentage of athletes categorized into each carrier profile is similar to what has been reported previously (Table 18): around 20% for persistent and non-carriers, and around 60% for intermittent carriers;[44,48] this, too, suggests misclassification was likely not a major concern in our analysis. Also similar to other studies, more males were found to be persistent carriers.[2,48]

Also, persistent carriers had higher odds of being colonized with SCC*mec* type IV MRSA than with other SCC*mec* types than intermittent carriers, though the same association was not true for USA300 strains. Given that all subjects who were colonized with PVL positive MRSA strains were intermittent carriers, the presence of PVL-encoding genes predicted the outcome perfectly, and thus, this could not be included in the multivariate logistic regression model. Additionally,

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persistent carriers were less likely to have oropharyngeal SA carriage only and more likely to have both nasal and oropharyngeal carriage than intermittent carriers. These results suggest that carrier profiles not only differ in their duration of colonization with SA, but also differ in the body sites that harbor SA and, potentially, in the type of SA isolates they are colonized with. Whether these associations remain true in other populations would require prospective studies in other groups.

Despite being a prospective study in a population with high prevalence of staphylococcal colonization, this study has some limitations. First, since medical history was mainly assessed through a baseline questionnaire, there is potential for recall bias. Some athletes might not have remembered that they, or a close contact, had a staphylococcal infection previously. Alternatively, they might remember having an infection but might not have recalled, or did not know what type of infection it was. A future study that includes a validation component comparing medical records to self-report could potentially assess the extent of this recall bias. In our study, to help athletes when answering this questionnaire, study personnel were present to answer any concerns the athletes might have had. Another limitation is the potential misclassification by categorizing athletes into contact or noncontact sports. The American Academy of Pediatrics (AAP) classifies sports as contact, limited-contact and noncontact sports;[13] given that only baseball is classified by the AAP as limited-contact, baseball was classified as a noncontact sport for this study, to reflect the less aggressive nature of the sport, as compared to contact sports. Nonetheless, if classifying baseball as noncontact would have lead to bias in the results observed, it would have probably overestimated the prevalence of colonization in noncontact sports, making the real difference between contact and noncontact sports even greater if baseball were excluded from the analyses. A sensitivity analysis classifying baseball players as contact instead of noncontact sports athletes (Table 30, Appendix B) did reveal a slightly stronger

association between contact sports and colonization with MRSA over time, making our original results more conservative, though still statistically significant. Additionally, not all athletes were swabbed every month; several individuals were absent during any given month's sampling, generating missing data. Using GLMM for assessing longitudinal colonization, however, only discards the missing observations without eliminating an individual with incomplete data. This modeling approach makes the best use of the available data, without discarding subjects with some missing data points. Results from two different sensitivity analyses, adjusting for the number of months subjects were swabbed and excluding subjects swabbed ≤5 months, were not materially different from our original estimates (Appendix B), indicating that missing data due to incomplete follow-up did not significantly alter our results.

Public Health Significance

The first reports of MRSA infections in athletes date back to 1998.[101,103] Since then, CA-MRSA has become the most common infectious agent reported to cause infections in sports participants, and skin and soft tissue infections are the most common infected site as well.[117,123] This study focused on the primary analysis of data collected from a prospective cohort of college student athletes over the course of two years. This research described the dynamics of staphylococcal colonization over time in a group considered at increased risk for CA-MRSA colonization and infection.

Longitudinal staphylococcal colonization was assessed, as well as the ecology of nasal and oropharyngeal colonization. The odds of colonization with MRSA and the odds of persistent colonization in collegiate athletes were also calculated. By analyzing the characteristics of MRSA colonizing isolates, we hope to provide some insights into the bacterial factors that differentiate

those microorganisms associated with colonization from those more likely to cause infection. Of previously reported infections in athletes, 99% have been caused by USA300 MRSA strains that carried the genes for PVL.[11,117] In our study, we also observed that most of the infecting MRSA isolates were USA300 and carried the genes to produce PVL.

Additionally, infections with MRSA are more costly and have longer lengths of hospitalization than MSSA infections. For athletes, these longer courses of infection would also mean longer absence from practice and competition, with the potential of decreasing their athletic performance. Furthermore, for college and high school athletes, these infections would also mean school absence, which could cause them fall behind in courses, and in turn, could jeopardize their participation in sports. Additionally, an outbreak among student athletes also has the potential to spread to their own academic community or to other academic institutions through competition and games. Even though the number of infections during our study was low, and given the risk of infection in colonized individuals, preventing colonization, or spread of colonization among sports athletes is potentially a cost effective preventive measure.

Understanding the dynamics and epidemiology of staphylococcal colonization in a group considered to be at high risk of colonization and infection will allow for improved surveillance of similar at-risk groups, development of new prevention strategies, such as selective decolonization of high-risk groups, preventive infection control measures during peak periods of colonization and infection, or selective decolonization of only specific strain types of CA-MRSA. Furthermore, a better understanding of the dynamics of staphylococcal colonization in this group of athletes at high-risk of staphylococcal colonization and infection is a step forward in understanding the epidemiology and dynamics of colonization in the general population.

Implications for the Field

Though extensive research has been done on staphylococcal infections and its risk factors, much of the work has been retrospective or based on hospital-derived populations. Thus, a gap still exists on knowledge about risk factors for staphylococcal colonization, and the dynamics of MSSA versus CA-MRSA colonization and infection in a healthy population.

This study was based on a prospective cohort of college athletes followed-up for two academic years. Over the two-years, athletes were screened monthly for colonization and strictly monitored to detect infections. With the analysis of these data, we hope to increase our understanding of the longitudinal colonization with CA-MRSA and the pathogenesis of CA-MRSA disease in an otherwise healthy population at high risk of staphylococcal colonization and infection. With this knowledge, we could help identify risk factors for colonization and disease, both related to the host and related to the pathogen. This increased knowledge can shed light on potential prevention and treatment strategies, which could be a first step in understanding the pathogenesis and epidemiology of CA-MRSA colonization, and preventing CA-MRSA carriage and infection in a broader population.

Innovation

Unlike previous studies that were retrospective or focused on hospital-based populations, our study has focused on a prospective cohort of college sports participants to assess nasal and oropharyngeal staphylococcal colonization during the course of two academic years, with monthly collection of colonization data. Though nasal colonization with *S. aureus* is a known risk factor for subsequent staphylococcal disease, a gap still exists on knowledge about risk factors for

staphylococcal colonization and the dynamics of MSSA versus CA-MRSA colonization and infection in a healthy population. Further, athletes – particularly those in contact sports – are at higher risk of infection with MRSA and have higher prevalence of nasal colonization than the general population: 5.4% vs. 1.3%.[10] However, most previous studies on athlete populations have focused on nasal colonization only during an outbreak setting. Thus, knowledge on the longitudinal dynamics of colonization, as well as on the role of colonization in other body sites on pathogenesis of infection is scarce.

Additionally, our study identified different carriage patterns and assessed the molecular characteristics of colonizing isolates, which have improved our understanding of the epidemiology of staphylococcal carriage and the pathogenesis of CA-MRSA disease. The knowledge gained from these studies may help improve surveillance strategies in similar high-risk groups and identify potential prevention strategies.

Future Directions

There is still no consensus on what would be the best strategy to prevent dissemination of colonizing and infecting staphylococcal strains in high-risk groups. Though decolonizing with mupirocin nasal ointment and chlorhexidine baths remain one of the most widely used and accepted interventions to stop infectious outbreaks, it is still controversial if these interventions could be beneficial in preventing future MRSA infections. Removing a colonizing isolate might lead to later colonization by a more virulent strain, which might not be as well adapted to its host and could potentially lead to illness in an otherwise healthy individual. On the other hand, for subjects who tend to get recurring infections due to their colonizing strain, decolonization could be a beneficial intervention, though later colonization after ending treatment will likely occur.

Applying the principle of bacterial interference could be another possibility. Colonization with a

non-virulent strain known as SA 502a could successfully prevent colonization and subsequent

infections with MRSA, and this strategy has been previously used in controlling outbreaks.[124-126]

Bacterial interference could thus be considered a potential preventive intervention, given the high

morbidity associated with MRSA infections.

Another issue to consider is whether prevention strategies need to be targeted only towards

colonized contact sports athletes, since they seem to be the ones at greater risk. So far, the best

practice to prevent MRSA and any infectious agent remains hand washing.[127]

To assess the effect of recall bias on the report of medical history, such as previous

staphylococcal infections in study participants and their contacts or previously taken antibiotics, a

future study could include a validation component, comparing medical records to self-report.

Also, whether the gender and racial differences in the prevalence of staphylococcal colonization

are due to biological factors of the host or due to behavioral or socioeconomic differences would

require a study with a more focused social and behavioral component.

Lastly, a larger study would likely be able to properly assess risk factors for infection in athletes

in a prospective manner. Such a study could be accomplished through a consortium of institutions

that include several athletic populations. If analyzing a collegiate population, an effort should be

made to collect information on students' housing arrangements to assess the effect of close

contact outside of the sports setting on the transmission of colonizing and infecting

staphylococcal isolates.

Conclusions

To sum up, colonization with SA was dynamic over time in this prospective study in healthy athletes. Colonization varied across seasons, reaching the highest prevalence around the time when these college athletes returned to practice after summer vacation. This high colonization prevalence could have been the cause of a small infectious outbreak experienced during the late summer months. Moreover, contact sports athletes were at increased risk of colonization than noncontact sports athletes, as demonstrated by higher odds of colonization over time, faster times to becoming colonized, and higher prevalence of intermittent and persistent staphylococcal carriage among contact sports athletes; also, these athletes are colonized with the epidemic clone, USA300, more frequently as well. Thus, these results suggest that contact sports athletes are the group where most prevention efforts and strategies should be placed to prevent staphylococcal colonization and subsequent SSTIs. Future studies should focus on identifying the most cost-effective prevention strategy in this high-risk group.

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Table 23. Characteristics of Reviewed Studies for Colonization in Athletes

Study	Study Design	Population	Gender	Category	Prior Intervention	Outbreak	Country	N	MSSA[a]	MRSA[a]
1. Creech CB, 2010[94]	Prospective	Football	Males	College	No	No	USA	100	0.24	0.09
2. Creech CB, 2010[94]	Prospective	Lacrosse	Females	College	No	No	USA	26	0.33	0.17
3. Oller AR, 2010[98]	Cross-sectional	Football	Males	College	No	No	USA	70	0.69	0.10
4. Oller AR, 2010[98]	Cross-sectional	Wrestling	Males	College	No	No	USA	25	0.64	0.04
5. Rackham DM, 2010[108]	Cross-sectional	Football	Males	College	No	No	USA	107		0.02
6. Rackham DM, 2010[108]	Cross-sectional	Soccer	Males	College	No	No	USA	14		0.14
7. Rackham DM, 2010[108]	Cross-sectional	Rowing	Females	College	No	No	USA	5		0.20
8. Rackham DM, 2010[108]	Cross-sectional	Baseball	Males	College	No	No	USA	32		0.00
9. Rackham DM, 2010[108]	Cross-sectional	Track	Males	College	No	No	USA	13		0.00
10. Rackham DM, 2010[108]	Cross-sectional	Wrestling	Males	College	No	No	USA	4		0.00
11. Rackham DM, 2010[108]	Cross-sectional	Basketball	Males	College	No	No	USA	3		0.00
12. Rackham DM, 2010[108]	Cross-sectional	Dance/cheer	Males	College	No	No	USA	2		0.00
13. Rackham DM, 2010[108]	Cross-sectional	Golf	Males	College	No	No	USA	2		0.00
14. Rackham DM, 2010[108]	Cross-sectional	Soccer	Females	College	No	No	USA	32		0.00
15. Rackham DM, 2010[108]	Cross-sectional	Dance/cheer	Females	College	No	No	USA	18		0.00
16. Rackham DM, 2010[108]	Cross-sectional	Track	Females	College	No	No	USA	15		0.00
17. Rackham DM, 2010[108]	Cross-sectional	Basketball	Females	College	No	No	USA	11		0.00
18. Rackham DM, 2010[108]	Cross-sectional	Volleyball	Females	College	No	No	USA	7		0.00
19. Rackham DM, 2010[108]	Cross-sectional	Swimming	Females	College	No	No	USA	4		0.00
20. Rackham DM, 2010[108]	Cross-sectional	Softball	Females	College	No	No	USA	3		0.00
21. Rackham DM, 2010[108]	Cross-sectional	Golf	Females	College	No	No	USA	1		0.00
22. Rackham DM, 2010[108]	Cross-sectional	Diving	Females	College	No	No	USA	1		0.00
23. Fontanilla JM, 2010[102]	Cross-sectional	Football and staff	Males	College	No	Yes	USA	118	0.53	0.04

Table 23, continued…

Study	Study Design	Population	Gender	Category	Prior Intervention	Outbreak	Country	N	MSSA[a]	MRSA[a]
24. Garza D, 2009[107]	Prospective	Football and staff	Males	Professional	No	No	USA	108	0.27	0.00
25. Stevens MP, 2008[128]	Cross-sectional	Basketball	Females	College	No	Yes	USA	13		0.31
26. Archibald LK, 2008[116]	Prospective	Football and staff	Males	College	Yes	Yes	USA	147	0.16	0.05
27. Muller-Premru M, 2005[120]	Cross-sectional	Football and staff	Males	N/A	No	Yes	Slovenia	12	0.17	0.25
28. Rihn JA, 2005[118]	Prospective	Football and staff	Males	High school	No	Yes	USA	102	0.31	0.03
29. Nguyen DM, 2005[25]	Cross-sectional	Football and staff	Males	College	No	Yes	USA	99	0.18	0.08
30. Kazakova SV, 2005[11]	Cross-sectional	Football and staff	Males	Professional	No	Yes	USA	84	0.42	0.00
31. Begier EM, 2004[96]	Cross-sectional	Football and staff	Males	College	No	Yes	USA	126	0.39	0.00
32. William JL, 2004[129]	Cross-sectional	Soccer	Males	High school	No	No	Malaysia/India	60	0.93	
33. Stacey AR, 1998[103]	Cross-sectional	Rugby	Males	N/A	No	Yes	UK	20	0.35	0.05
34. Lindenmayer JM, 1998[101]	Cross-sectional	Wrestling	Males	High school	No	Yes	USA	32		0.09
35. Romano R, 2006[119]	Retrospective	Football	Males	College	Yes	Yes	USA	106	0.18	0.07

[a]: These numbers represent proportions.

Sensitivity Analyses

Table 24. Frequency of demographics and medical history for a cohort of 377 collegiate athletes by the number of times they were swabbed

Variable	Total Cohort N=377 N (%)	Swabbed ≤5 Months N=112 (29.71) N (%)	Swabbed >5 and <13 Months N=162 (42.97) N (%)	Swabbed ≥13 Months N=103 (27.32) N (%)
Categorized Sports				
Noncontact	153 (40.58)	80 (71.43)	60 (37.04)	13 (12.62)
Contact	224 (59.42)	32 (28.57)	102 (62.96)	90 (87.38)
Gender				
Female	161 (42.71)	51 (45.54)	72 (44.44)	38 (36.89)
Male	216 (57.29)	61 (54.46)	90 (55.56)	65 (63.11)
Race/Ethnicity				
Caucasian	280 (74.27)	87 (77.68)	119 (73.46)	74 (71.84)
African-American	80 (21.22)	17 (15.18)	38 (23.46)	25 (24.27)
Other	17 (4.51)	8 (7.14)	5 (3.09)	4 (3.88)
College Year				
Freshman	149 (39.52)	44 (39.29)	67 (41.36)	38 (36.89)
Sophomore	87 (23.08)	21 (18.75)	29 (17.90)	37 (35.92)
Junior	70 (18.57)	14 (12.50)	34 (20.99)	25 (21.36)
Senior	59 (15.65)	32 (28.57)	22 (13.58)	5 (4.85)
Trainers	12 (3.18)	1 (0.89)	10 (6.17)	1 (0.97)
History of SA infection				
No	355 (94.16)	107 (95.54)	150 (92.59)	98 (95.15)
Yes	22 (5.84)	5 (4.46)	12 (7.41)	5 (4.85)
SA infection in contacts				
No	261 (69.23)	90 (80.36)	107 (66.05)	64 (62.14)
Yes	116 (30.77)	22 (19.64)	55 (33.95)	39 (37.86)
Previous surgeries				
No	275 (72.94)	74 (82.22)	137 (68.50)	64 (73.56)
Yes	102 (27.06)	16 (17.78)	63 (31.50)	23 (26.44)

Table 24, continued…

Variable	Total Cohort N=377 N (%)	Swabbed ≤5 Months N=112 (29.71) N (%)	Swabbed >5 and <13 Months N=162 (42.97) N (%)	Swabbed ≥13 Months N=103 (27.32) N (%)
Comorbidities				
No	362 (96.02)	110 (98.21)	153 (94.44)	99 (96.12)
Yes	15 (3.98)	2 (1.79)	9 (5.56)	4 (3.88)
Antibiotics in Previous 6 Months				
No	329 (87.27)	100 (89.29)	137 (84.57)	92 (89.32)
Yes	48 (12.73)	12 (10.71	25 (15.43)	11 (10.68)
Ever SA				
No	90 (23.87)	48 (42.86)	27 (16.67)	15 (14.56)
Yes	287 (76.13)	64 (57.14)	135 (83.33)	88 (85.44)
Ever MRSA				
No	202 (53.58)	76 (67.86)	77 (47.53)	49 (47.57)
Yes	175 (46.42)	36 (32.14)	85 (52.47)	54 (52.43)
Carrier Profile				
Never	90 (23.87)	48 (42.86)	27 (16.67)	15 (14.56)
Intermittent	200 (53.05)	29 (25.89)	101 (62.35)	70 (67.96)
Persistent	87 (23.08)	35 (31.25)	34 (20.99)	18 (17.48)

Aim #1

Table 25. Odds ratios for current staphylococcal carriage as a function of previous staphylococcal carriage in 377 subjects with 2,405 observations, based on multinomial mixed models with random intercept

Colonization on Previous Month	Model 1 n=2,405		Model 2 n=2,405		Model 3 n=2,405	
	OR	95% CI	OR	95% CI	OR	95% CI
Not colonized on previous month	1.00	---	1.00	---	1.00	---
Nasal and oropharyngeal on previous month						
Nasal and oropharyngeal	6.97	4.17, 11.66	6.88	4.11, 11.52	7.19	4.19, 12.34
Nasal only	2.00	1.18, 3.37	1.99	1.18, 3.36	1.82	1.04, 3.17
Oropharyngeal only	4.28	2.85, 6.43	4.30	2.87, 6.45	4.15	2.73, 6.31
Nasal only on previous month						
Nasal and oropharyngeal	26.04	16.45, 41.21	25.83	16.31, 40.92	26.39	16.23, 42.91
Nasal only	20.18	13.63, 29.88	20.18	13.63, 29.88	18.33	12.09, 27.79
Oropharyngeal only	1.77	1.03, 3.04	1.77	1.03, 3.04	1.57	0.87, 2.83
Oropharyngeal only on previous month						
Nasal and oropharyngeal	63.55	36.52, 110.61	61.62	35.41, 107.25	64.34	36.05, 114.82
Nasal only	31.45	19.98, 49.52	31.20	19.81, 49.13	29.06	18.06, 46.76
Oropharyngeal only	7.64	4.58, 12.76	7.55	4.52, 12.59	7.55	4.44, 12.85
Month (time)						
Nasal and oropharyngeal	0.98	0.95, 1.01	0.98	0.95, 1.01	0.98	0.95, 1.01
Nasal only	0.99	0.96, 1.01	0.99	0.96, 1.01	0.98	0.96, 1.01
Oropharyngeal only	1.03	1.01, 1.06	1.03	1.00, 1.06	1.03	1.01, 1.06

[a]: To be included in the model, both current and previous month variables could not be missing: from 3,291 observations, only 2,405 were included in the analysis.

[b]: Adjusted for contact sport (contact vs. noncontact), gender, race, college year

Model 1: Original model

Model 2: Adjusting for the number of months athletes were swabbed

Model 3: Excluding subjects swabbed 5 times or less

Table 26. Odds ratios for crude and adjusted multinomial mixed models with random intercept to assess the association between seasons and staphylococcal colonization: Full cohort

	Model 1 n=3,291		Model 2 n=3,291		Model 3 n=2,873	
	OR	95% CI	OR	95% CI	OR	95% CI
Calendar Season						
Winter	1.00	---	1.00	---	1.00	---
Spring						
MRSA	1.52	0.86, 2.70	1.53	0.86, 2.71	1.67	0.93, 3.01
MSSA	0.85	0.52, 1.41	0.85	0.51, 1.41	0.87	0.52, 1.47
Summer						
MRSA	1.70	1.23, 2.35	1.69	1.23, 2.34	1.62	1.16, 2.28
MSSA	1.38	1.05, 1.82	1.37	1.04, 1.80	1.49	1.12, 1.98
Fall						
MRSA	0.45	0.32, 0.62	0.44	0.32, 0.61	0.47	0.34, 0.66
MSSA	0.89	0.70, 1.13	0.88	0.69, 1.13	0.89	0.69, 1.14

Model 1: Original model
Model 2: Adjusting for the number of months athletes were swabbed
Model 3: Excluding subjects swabbed 5 times or less

Table 27. Odds ratios for crude and adjusted multinomial mixed models with random intercept to assess the association between seasons and staphylococcal colonization: Football team only

	Model 1 n=1,362		Model 2 n=1,362		Model 3 n=1,273	
	OR	95% CI	OR	95% CI	OR	95% CI
Calendar Season						
Winter	1.00	---	1.00	---	1.00	---
Summer						
MRSA	1.52	0.99, 2.35	1.53	0.99, 2.36	1.54	1.00, 2.38
MSSA	1.69	1.15, 2.50	1.70	1.16, 2.52	1.73	1.17, 2.56
Fall						
MRSA	0.48	0.30, 0.76	0.48	0.30, 0.76	0.49	0.31, 0.78
MSSA	1.30	0.89, 1.90	1.31	0.90, 1.92	1.31	0.89, 1.91
Athletic Season						
Football season	1.00	---	1.00	---	1.00	---
Post-season						
MRSA	1.39	0.90, 2.14	1.38	0.89, 2.14	1.46	0.93, 2.27
MSSA	0.91	0.64, 1.30	0.91	0.64, 1.29	0.99	0.69, 1.43
Pre-season						
MRSA	3.18	1.97, 5.13	3.18	1.97, 5.13	3.37	2.06, 5.52
MSSA	1.31	0.88, 1.96	1.32	0.88, 1.96	1.52	1.00, 2.30
Spring training						
MRSA	1.35	0.75, 2.43	1.34	0.75, 2.42	1.37	0.76, 2.47
MSSA	0.34	0.19, 0.63	0.34	0.19, 0.63	0.36	0.20, 0.67

Model 1: Original model
Model 2: Adjusting for the number of months athletes were swabbed
Model 3: Excluding subjects swabbed 5 times or less

Table 28. Hazard ratios for crude and adjusted models

	All athletes			Freshmen only		
	Model 1 n=186	Model 2 n=186	Model 3 n=122	Model 1 n=79	Model 2 n=79	Model 3 n=52
	HR (95% CI)	HR (95% CI)	HR (95% CI)	HR (95% CI)	HR (95% CI)	HR (95% CI)
Noncontact sports	1.00 ---	1.00 ---	1.00 ---	1.00 ---	1.00 ---	1.00 ---
Contact sports	1.61 1.02, 2.55	1.58 0.95, 2.61	1.42 (0.64, 2.61)	1.89 (1.04, 3.44)	2.03 (1.17, 3.68)	1.52 (0.77, 2.99)

Model 1: Original model
Model 2: Adjusting for the number of months athletes were swabbed
Model 3: Excluding subjects swabbed 5 times or less
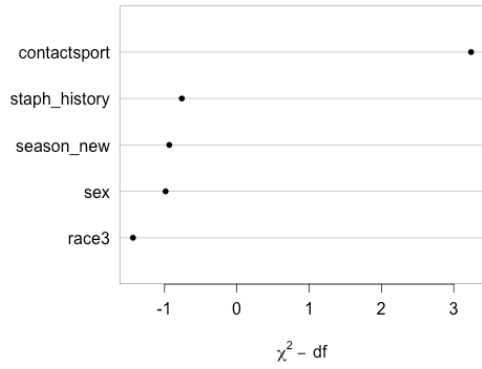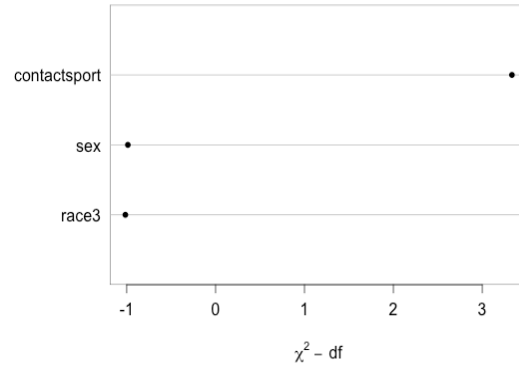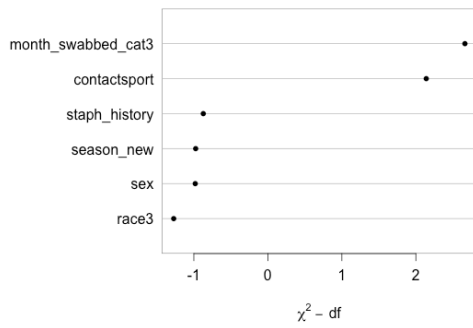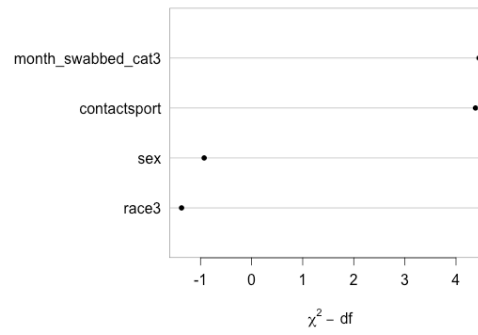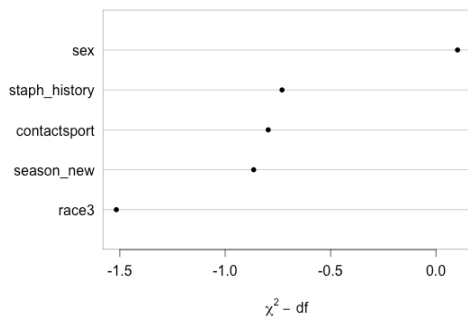
Figure 17. Contribution of each covariate in explaining the hazards of becoming colonized with SA. A, C and E: models include athletes from all college years. B and D: models include only freshmen. E: excludes subjects swabbed only 5 months or less.

Aim #2

Table 29. Odds ratios for unadjusted multinomial mixed models with random intercept

	Model 1 n=3,291		Model 3 n=2,873		Model 4 n=3,291	
	OR	95% CI	OR	95% CI	OR	95% CI
Noncontact sports	1.00	---	1.00	---	1.00	---
Contact sports						
MRSA	2.25	1.14, 4.43	3.30	1.50, 7.26	4.38	2.08, 9.22
MSSA	1.68	1.06, 2.65	1.47	0.85, 2.54	1.80	1.11, 2.93

Model 1: Original model
Model 3: Excluding subjects swabbed 5 times or less
Model 4: categorizing baseball as a contact sport

Table 30. Odds ratios for adjusted multinomial mixed models with random intercept

	Model 1 n=3,291		Model 2 n=3,291		Model 3 n=2,873		Model 4 n=3,291	
	OR	95% CI	OR	95% CI	OR	95% CI	OR	95% CI
Noncontact sports	1.00	---	1.00	---	1.00	---	1.00	---
Contact sports								
MRSA	2.36	1.13, 4.93	3.08	1.36, 6.99	3.59	1.50, 8.62	3.79	1.68, 8.52
MSSA	1.57	0.94, 2.62	1.57	0.89, 2.77	1.33	0.71, 2.50	1.67	0.95, 2.92
Male	1.00	---	1.00	---	1.00	---	1.00	---
Female								
MRSA	0.26	0.13, 0.50	0.25	0.13, 0.48	0.42	0.19, 0.90	0.36	0.17, 0.75
MSSA	0.80	0.50, 1.28	0.79	0.49, 1.26	1.15	0.66, 2.00	0.91	0.55, 1.53
Caucasian	1.00	---	1.00	---	1.00	---	1.00	---
African-American								
MRSA	0.35	0.15, 0.79	0.34	0.15, 0.78	0.38	0.17, 0.86	0.33	0.15, 0.72
MSSA	0.77	0.44, 1.34	0.76	0.43, 1.35	0.78	0.43, 1.43	0.79	0.46, 1.38
Other race/ethnicity								
MRSA	0.46	0.09, 2.31	0.40	0.08, 2.02	0.18	0.02, 1.53	0.41	0.08, 2.04
MSSA	1.39	0.47, 4.15	1.47	0.49, 4.40	2.73	0.76, 9.85	1.40	0.47, 4.13
Freshman	1.00	---	1.00	---	1.00	---	1.00	---
Sophomore								
MRSA	1.25	0.53, 2.79	1.40	0.63, 3.13	1.22	0.54, 2.75	1.24	0.56, 2.75
MSSA	1.79	1.02, 3.14	1.82	1.03, 3.22	1.61	0.88, 2.95	1.74	0.99, 3.04
Junior								
MRSA	1.25	0.54, 2.90	1.26	0.55, 2.90	1.33	0.58, 3.07	1.27	0.55, 2.92
MSSA	1.69	0.94, 3.04	1.67	0.92, 3.01	2.04	1.09, 3.82	1.71	0.95, 3.07
Senior								
MRSA	1.07	0.40, 2.81	0.84	0.31, 2.29	0.89	0.29, 2.79	1.10	0.42, 2.86
MSSA	1.20	0.61, 2.37	1.21	0.60, 2.46	1.38	0.60, 3.19	1.23	0.63, 2.40
Trainers								
MRSA	0.97	0.14, 6.69	1.06	0.15, 7.37	1.67	0.25, 11.15	1.54	0.22, 10.86
MSSA	0.82	0.21, 3.27	0.74	0.18, 3.01	0.73	0.17, 3.13	0.90	0.22, 3.65

Table 30, continued…

	Model 1 n=3,291		Model 2 n=3,291		Model 3 n=2,873		Model 4 n=3,291	
	OR	95% CI	OR	95% CI	OR	95% CI	OR	95% CI
No previous SA infections	1.00	---	1.00	---	1.00	---	1.00	---
Previous SA infections								
MRSA	5.35	1.51, 18.87	5.41	1.55, 18.87	7.75	2.30, 26.32	5.38	1.54, 18.77
MSSA	2.19	0.86, 5.52	2.09	0.83, 5.29	2.89	1.10, 7.63	2.14	0.85, 5.40
No SA infections in contacts	1.00	---	1.00	---	1.00	---	1.00	---
SA infections in contacts								
MRSA	0.45	0.22, 0.94	0.47	0.23, 0.98	0.55	0.26, 1.13	0.44	0.21, 0.90
MSSA	0.72	0.43, 1.20	0.71	0.43, 1.19	0.75	0.43, 1.31	0.73	0.44, 1.20
No comorbidities	1.00	---	1.00	---	1.00	---	1.00	---
Any comorbidities								
MRSA	2.90	0.65, 13.01	2.91	0.66, 12.81	3.35	0.83, 13.58	3.21	0.73, 14.18
MSSA	1.71	0.59, 5.01	1.67	0.57, 4.87	1.87	0.62, 5.61	1.67	0.58, 4.88
No previous surgeries	1.00	---	1.00	---	1.00	---	1.00	---
Previous surgeries								
MRSA	1.28	0.63, 2.60	1.31	0.65, 2.63	1.33	0.66, 2.68	1.30	0.65, 2.61
MSSA	1.31	0.80, 2.15	1.29	0.79, 2.12	1.27	0.75, 2.15	1.35	0.83, 2.21
No previous antibiotics	1.00	---	1.00	---	1.00	---	1.00	---
Previous antibiotics								
MRSA	1.29	0.50, 3.34	1.30	0.51, 3.33	1.63	0.64, 4.20	1.40	0.54, 3.59
MSSA	0.59	0.30, 1.18	0.60	0.30, 1.19	0.66	0.31, 1.38	0.61	0.30, 1.20

Model 1: Original model
Model 2: Adjusting for the number of months athletes were swabbed
Model 3: Excluding subjects swabbed 5 times or less
Model 4: categorizing baseball as a contact sport

Aim #3

Table 31. Adjusted multinomial logistic regression models comparing the odds of being persistent, intermittent or never SA carriers between contact and noncontact sports athletes

	Model 1 n=377		**Model 2** n=377		**Model 3** n=265	
	OR	**95% CI**	**OR**	**95% CI**	**OR**	**95% CI**
Contact sports						
Never SA carriers	1.00	---	1.00	---	1.00	---
Intermittent SA carriers	3.60	2.02, 6.40	1.82	0.91, 3.65	1.96	0.97, 3.98
Persistent SA carriers	2.39	1.21, 4.72	2.38	1.08, 5.25	2.27	0.93, 5.54

Table 32. Logistic regression models comparing the odds of being persistent or intermittent SA carriers by isolate type between 287 athletes (excluding those who were never SA carriers)

	Model 1 n=287		**Model 2** n=287		**Model 3** n=223	
	OR	**95% CI**	**OR**	**95% CI**	**OR**	**95% CI**
USA300 vs. other						
Intermittent SA carriers	1.00	---	1.00	---	1.00	---
Persistent SA carriers	0.68	0.30, 1.56	0.88	0.38, 2.02	1.00	0.42, 2.39
SCC*mec* IV vs. other						
Intermittent SA carriers	1.00	---	1.00	---	1.00	---
Persistent SA carriers	5.30	2.88, 9.77	6.31	3.13, 12.72	5.15	2.38, 11.17

Table 33. Odds ratios for exclusive nasal staphylococcal carriage in persistent and intermittent staphylococcal carriers by colonization site in 287 subjects, based on multinomial mixed models with random intercept (excluding those who were never colonized)

Colonization Site/Type	**Model 1** n=1,433		**Model 2** n=1,433		**Model 3** n=1,253	
	OR	**95% CI**	**OR**	**95% CI**	**OR**	**95% CI**
Intermittent carriers	1.00	---	1.00	---	1.00	---
Persistent carriers						
Nasal and oropharyngeal	2.05	1.40, 3.02	2.13	1.42, 3.18	2.29	1.50, 3.49
Oropharyngeal only	0.16	0.08, 0.31	0.16	0.08, 0.31	0.18	0.09, 0.38